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Grouping of Legumes According to Biological Reactions of Their Seed Proteins - I. L. Baldwin, E. B. Fred, and E. G. Hastings 217

Behavior of Certain Fern Prothallia under Prolonged Cultivation David M. Mottier 244

Ecology of Fungi in the Chicago Region. Contributions from the Hull Botanical Laboratory 367 - - - - - - V. O. Graham 267

Origin and Development of Tissues in Rhizome of Pteris aquilina Contributions from the Hull Botanical Laboratory 368 - C. Y. Chang 288

Relation between Fruit Size and Abscission of Young Orange Fruits A. R. C. Haas 307

Briefer Articles

The Auximone Theory - - - - - Florence A. Mockeridge 314

Staining of Yeast Cells - - Arthur P. Kelley and M. B. Shoemaker 318

Current Literature

Book Reviews - - - - - - - - - - - - - 319

Notes for Students - - - - - - - - - - - - 323

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Volume LXXXIII Number 3

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

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April and May 1927

Humanizing the Sciences

Comment on *The Nature of the World and of Man* by sixteen members of the Faculty of the University of Chicago is running high.

Whatever their intentions toward college Freshmen, which are of no very great interest to the world outside the universities, the sixteen scientists have written sixteen fireside lectures ranging from astronomy to psychology, which the critics rank as the best of recent attempts to humanize the sciences. Opinion runs as follows:

"One of the finest and most engrossing popularizations of current science in existence. . . . . It is simple, clear, concrete, reliable. One emerges from it the richer by solid information and definite concepts."—HENRY HAZLITT, *New York Sun*.

"A manual of the physical and biological sciences of which it would be hard to speak too highly. The volume is so many things that an outline ought to be but frequently is not. It is selective in the sense of plucking the real heart out of a body of data."—*New York Times Book Review*.

"It is unexcelled for the general reader and many of those who have had scientific training will here learn for the first time what it is all about. Above all, it is vigorously inspiring."—*New York Herald-Tribune*.

"For the story is well told, well illustrated, and well colored with human significance. Certainly the volume is accurate; the names of the various contributors guarantee that. Certainly it is interesting and readable, and popular without being diluted."—*The Nation*.

"It sweeps on from one great truth to another in a way that gives one a profound respect for the recent progress of science."—*Saturday Review of Literature*.

"It is fascinating reading for anyone who has a spark of that divine curiosity about the ways of life which causes man to seek, and seeking, to progress."—*Chicago Tribune*.

That the orientation course upon which *The Nature of the World and of Man* is based is helping the students build a workable philosophy of life is indicated in a large body of comment from these students themselves. The following statements are representative of the student attitude toward the course:

"To me this course has been most stimulating. It has given a new and broader conception of the universe. It has aroused in me a new interest and spirit of curiosity. I feel that to some extent, I have learned to value the scientific spirit. The influence that science has upon our lives is better appreciated. I believe that the course was intended to present a brief but comprehensive picture of everything that concerns us, to shake up our minds, and to stimulate us to a little thought. It has done those things for me. I am eager to take more science courses and find out how and why the universe is as it is."

"I find the spirit of science to be open-minded, strong, fearless, and wholesome. It is strange to consider one's self related to the great universe of things. I am very enthusiastic about the course. The realization or the apprehension of the infinitude of the heavens, the universe of the atom has put me in my proper place in the cosmos."

"This course is called an orientation course. This expresses well its aim and what it has

done for me. It has taken me out of the universe and has set me at a point outside where I could see everything that was going on and look at the ways of the universe from an impartial perspective. It has given an introduction to the natural sciences and their interrelationship. The greatest benefit I have received, a benefit which sinks all other results into insignificance, is that it has caused me to ask myself such questions as 'Who am I?' 'What am I?' and 'Where am I?' It has changed my outlook on the world."

Two printings of *The Nature of the World and of Man* have already been exhausted and a new printing of 15,000 has been ordered. This will include a revision of the chapter on "The Nature of Chemical Processes" by Julius Stieglitz. As re-written it will simplify the subject of chemistry for the layman. *THE NATURE OF THE WORLD AND OF MAN.* By Sixteen Members of the Faculty of the University of Chicago. *$4.00, postpaid $4.15.*

* * *

Gangs

Gang activities are familiar enough in cosmopolitan cities, but strangely, there has been no adequate sociological study of them. The forces that explain gangdom, adult and juvenile, have been brought to light by Professor Frederic M. Thrasher in *The Gang*, a book which critics believe destined to become the standard source book on the subject.

Professor Thrasher spent six years in his study of gangland—the poverty belt which surrounds Chicago's loop district. He lived with the gangsters, interviewed their leaders, and became intimately acquainted with gang life from the inside. Mingling with the "Murderers," the "Deadshots," and members of hundreds of other gangs, he learned their mysteries and secret signs, their attitudes toward society, and toward each other. And he found in investigating 1,313 gangs of all ages and sizes, the facts which explain the sensational features of gang life which are flaunted in the headlines.

He looks upon the gang as a social makeshift, which naturally appears in the cracks of the community and inevitably fosters the spread of crime. Abandoning the idea of a gang instinct, he attempts through a careful study of the natural history, structure, and functioning of the gang, to arrive at the true causes for its existence.

Mr. Thrasher has collected a vast amount of intensely interesting material which he discusses with a minimum of seminar jargon. A large part of the case material was secured from the gang boys themselves. A typical case is the following, obtained in an interview with a gang boy:

Billy, the Brains

Billy was the brains of the gang. He was "educated," a high-school boy too. He would work sometimes, but not often. The kids would bring their "stuff" to him. One day we had a big fight over it when we were robbing a merchandise car; we had cigarettes, pop, and a lot of other stuff. Billy had his stuff put away in a box with straw on top of it. The watchman looked at the straw, but Billy told him it was for a rabbit. Billy would sell "cartoons" (of cigarettes) for a half a dollar apiece.

Billy would plan things for our gang. He would get us a place to sleep when we were bumming away from home. He would get us keys to the bread boxes, so that we could get food when we were hungry. We would get the bread after the bakers left it early in the morning before the stores opened. . . . . Billy would find us a place to sleep in some house or basement. He would go around everywhere to see if there was a place to sleep or rob; he was a regular investigator.

Henry Zorbaugh of New York University comments: "Dr. Thrasher's monograph should be of interest to the sociologist and psychologist as a model of social research and a contribution to our knowledge of human behavior; to educator and social worker as an illuminating background against which to consider the practical problems of boy adjustment; and to the romantically minded as a fascinating story of 'the web of life' as it is spun in a great city."

Harry Hansen says in the *New York World:* "Here is a remarkable document and one that ought to be a source book for years to

come. . . . . He has dug right down into boyland, gone to the root of the problem, and produced a book that will become an authority." *THE GANG*. By FREDERIC M. THRASHER. *$3.00, postpaid $3.15*.

* * *

Ernest DeWitt Burton

During a half-century of research Ernest DeWitt Burton achieved an international reputation as a New Testament scholar. In two years of extraordinary activity as President of the University of Chicago, he became equally well known as an educational executive. In addition, a leader in missionary and denominational enterprises, he offers an inspiring example of life lived to the full.

Dr. Burton's personality made a deep and lasting impression on those who knew him. "In him," says Henry Justin Smith, "were concentrated the mental vigor of youth and the benignity of age; the devotion of a missionary and the acuteness of a worldling; the simplicity of a plain man and the delicacy of a true aristocrat."

Of his associates, no one is better qualified to portray this great and enormously attractive individual than Thomas W. Goodspeed, who has been an intimate witness of his life-activity. In *Ernest DeWitt Burton: A Biography*, Mr. Goodspeed has followed his career from his student days at Denison, through his work as head of the Oriental Educational Commission, chairman of the China Educational Commission, and President of the University of Chicago, to his last days.

Mr. Goodspeed has not attempted to idealize Ernest DeWitt Burton, but to present him as he was in every period of his life. *ERNEST DeWITT BURTON*. By THOMAS W. GOODSPEED. *$3.00, postpaid $3.10*.

*

Dr. Burton left unpublished some exceedingly valuable work, and this has been preserved in two posthumous volumes, *Christianity in the Modern World*, and *A Short Introduction to the Gospels*.

The first of these volumes brings out one of the most attractive phases of Dr. Burton's character. Internationally known as a New Testament scholar, and as President of the University of Chicago, Ernest DeWitt Burton has another side no less great, but not as well known. This was Dr. Burton the Christian and exemplar of the practical Christian life, the man of deep religious experience and conviction.

This phase of his character is uppermost in this collection of his fugitive utterances on religious themes. Here are his great *apologia pro vita sua*, "Why I am content to be a Christian"; his views upon Jesus and Paul; and the essence of his thinking on Christian education and world-problems.

This book is fittingly concluded with "Christian Education in China," Dr. Burton's last public address.

The second of the two volumes incorporates his final conclusions in a work representing one of his most important projects. Dr. Burton continued during his last years the researches concerning the origins of the Four Gospels which had been the basis of his valued *Short Introductions to the Gospels*. He made further investigations regarding the dates of the Synoptic Gospels, and approached the Fourth Gospel from a new point of view, applying the method of source criticism which he had already used with conspicuous success in the case of the Synoptic Gospels.

These significant advances have been incorporated by Harold R. Willoughby in a new revision of the volume, which brings to biblical students valuable new material. Two important sections of fresh material found in Dr. Burton's files have been added. The first renders an exceptional judgment regarding the dates of the Synoptic Gospels. The second outlines a unique theory concerning the composition of the Fourth Gospel which illuminates the Johannine depiction of Jesus.

This authoritative revision places before students of the Gospels those facts concerning the purpose and point of view of each of them which are most necessary for an intelligent understanding of them. *CHRISTIANITY IN THE MODERN WORLD*. By ERNEST D. BURTON. *$2.00, postpaid $2.10*. *A SHORT INTRODUCTION TO THE GOSPELS*. By ERNEST D. BURTON AND HAROLD R. WILLOUGHBY. *$1.75, postpaid $1.85*.

Teaching Science in the Schools

By

ELLIOT R. DOWNING

Scientific study of science teaching has had some valuable results, and these Mr. Downing summarizes in this volume. Here is shown how the growing body of facts about the pedagogy of science, which have been ascertained by the experimental method, may be applied practically to the problems of the science teacher.

This book describes the social and economic background of the science-teaching movement, the history of science teaching in our elementary and secondary schools, present conditions in science teaching, and the aims held.

From this point Mr. Downing takes up the principles of selection and organization of science materials, and actual methods of instruction—by book, lecture-demonstration, and laboratory. There is specific information on notebooks and drawings, field trips, testing results; and suggestions are made for a complete science library in the school.

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The new "University of Chicago Junior College Series" of German texts embodies all the most recent progress in the methods

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Two volumes are ready for immediate use. They are *A Modern German Grammar*, by Peter Hagboldt and F. W. Kaufmann; and *Inductive Readings in German*, Book I, by the same authors.

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The "University of Chicago Junior College Series" of French texts, initiated not long ago with Otto F. Bond's *The Sounds of French* and *An Introduction to the Study of French*, has, because of the departure of its methods from those generally in use, become a center of controversy. Mr. Bond maintains that most of his critics have mistaken his aims. Answering one of them, Colley F. Sparkman, in the *Modern Language Journal*, he says: "I am afraid that Mr. Sparkman suspects me of having wished upon the public a substitute for a composition grammar or some *bête difforme* lacking synthetic legs and a *raison d'être*, since he would have me add various types of exercises involving composition. Let me point out that the Preface (page viii) states the purpose of the book as being to enable the student to read (not write or converse!) in a short time and to prepare him for better and more rapid progress in acquiring expression power.

"Incidentally, why do language teachers struggle manfully to uphold the conversation and composition banner in beginning courses, since better than 50 per cent of their beginners leave them when the requirements have been satisfied?"

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By C. JUDSON HERRICK

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BOTANICAL GAZETTE

May 1927

GROUPING OF LEGUMES ACCORDING TO BIOLOGICAL REACTIONS OF THEIR SEED PROTEINS[1]

POSSIBLE EXPLANATION OF PHENOMENON OF CROSS INOCULATION

I. L. BALDWIN, E. B. FRED, AND E. G. HASTINGS

(WITH TWO FIGURES)

Introduction

Among the root nodule bacteria of leguminous plants a number of races have developed, each capable of causing the formation of nodules on the roots of certain species of the Leguminosae and not on others. The leguminous plants, on the basis of their relation to the root nodule bacteria, have been divided into "cross inoculation" groups, any member of which may be infected by the bacteria from any other member of the group.

The adaptations of the various races of the root nodule bacteria to the different species of the Leguminosae and the phenomenon of cross inoculation have occasioned speculation and study since 1891, when NOBBE and his co-workers (**26**) discovered that the organism of pea nodules is unable to form nodules on the roots of serradella and lupine.

Determination of these cross inoculation groups is of great practical importance in the culture of leguminous plants, and such studies have been made of practically all the cultivated legumes.

[1] Contribution from the department of agricultural bacteriology, University of Wisconsin.

On the other hand, the factors responsible for or controlling the adaptation of the various races of nodule bacteria to the specific host plants present a question of scientific interest which has not received careful study. While these groups of bacteria possess differences in their adaptations to the host plants, they are almost indistinguishable by the common bacteriological methods.

Generally speaking, the species belonging to any cross inoculation group represent those closely related botanically; however this is not true in all cases. In the group commonly spoken of as the cowpea group, there are at least 10 genera represented.

Why should the organism responsible for nodule formation on the roots of the lima bean be able to infect the roots of the cowpea and not those of the garden bean? The answer must be found in the differences among the various species of the host plant and the various races of the root nodule organism. And, inasmuch as the question is not one of the formation or lack of formation of root nodules on a particular plant species, but rather one of the adaptation of a certain bacterial race to the particular plant species, it would seem probable that the chemical phases of the problem are of more importance than the morphological ones.

A similar problem is presented in a study of the factors which render certain varieties of a plant species resistant to invasion by a plant parasite, while other varieties of the same species are susceptible to attack by the same parasite. Since this problem is one of the ability or lack of ability on the part of the plant to withstand the attack of the parasite, it is conceivable that either or both physiological and morphological factors in the plant may play a part. Investigations in this field indicate that in some cases the physiological conditions are the controlling factors, while in others morphological variations seem to determine the resistance or susceptibility to the attack of the parasite.

DICKSON and HOLBERT (9), working on the resistance of the corn plant to root and stalk rot, found that the resistance or susceptibility of the corn plant was connected with the rate and type of carbohydrate metabolism. NELSON (25), by serological methods, was able to distinguish between the proteins of a wilt-resistant flax and a non-resistant variety. WILLAMAN, PERVIER, and TRIEBOLD (41)

studied the resistance of certain varieties of plums to attack by *Sclerotinia cinerea*, and could find no significant differences between the physiology of the resistant and susceptible varieties, but did find differences in the physical character of the fruit which they believed to be responsible for the differences in the resistance of the host plant to invasion by the parasite. Many other similar examples might be mentioned.

An interesting parallelism to the grouping of the legumes by the root nodule organisms is furnished by the grouping of the legumes by many of the plant parasites. GARDNER (**11**) reported that *Bact. vignae* which causes the bacterial spot disease of cowpea also attacks catjang, adzuki bean, velvet bean, hyacinth bean, and tick trefoil, all of which belong in a single cross inoculation group.

Relatively few data are available to explain the adaptation of the root nodule bacteria to a particular plant species. SIMON (**32**) suggested that the protein characteristics of the plant may explain the adaptation of bacteria to the plant. No experimental data on this point are given, and a study of the later literature fails to reveal any further communications relative to the subject. FRED and DAVENPORT (**10**), in a study of the influence of reaction on the root nodule bacteria and by a comparison with the results of other workers upon the influence of reaction upon the leguminous plants, found a correlation between the two.

During the past two decades considerable effort has been expended in the determination of the so-called natural system of plant grouping or classification of serological methods. Inasmuch as the fragmentary work, which had been carried out with legumes, checks with the groupings established by cross inoculation, it was felt advisable to repeat and extend this work to include all the more commonly cultivated legumes.

Plant groups as determined by serological methods

In studying plant relationships at least five different serological reactions have been utilized: (1) precipitin, (2) complement fixation, (3) anaphylaxis, (4) conglutinin, and (5) Abderhalden. Of these, the precipitin reaction was the first to be used in the study of plant relationships, and has been more generally used than any of the others.

The complement fixation, anaphylaxis, and conglutinin reactions, however, have all proved valuable in this connection. The Abderhalden reaction has not been found suitable for this work.

The work with the higher plants may be divided into two groups. Certain workers have been interested in the possibility of differentiating between various plants, while others have attempted to bring together into groups closely related ones. The attempt to separate one plant from another by serological methods dates back to KOWARSKI (**19**), who in 1901 was able to distinguish between wheat, rye, oats, and peas by the precipitin reaction. Many workers have since found it possible to differentiate between species by the various serological reactions.

RELANDER (**29, 30**) was probably the first to demonstrate that the technique of the reactions can be so adjusted as to enable differences to be noted between varieties within a species. He was able to differentiate between American, Norwegian, Finnish, and Italian red clovers, and between 2-rowed and 6-rowed barley by the use of the precipitin test. BALLNER and BURROWS (**4**) demonstrated small differences between strains in both beans and peas by the use of the complement fixation reaction.

MAGNUS and FRIEDENTHAL probably were the first to use serological methods in a study of plant relationships. In their first work (**22**) they investigated the relationships between certain fungi. In a later paper (**23**) they reported the results of work with antigens secured from the higher plants. In this connection they stated: "The important discovery was made, that the duration of the immunization had an important influence not only quantitatively but also qualitatively, in that with longer immunization the degree of relationship always assumed wider limits." MAGNUS (**21**), working with members of the grass family, suggested that the best method of establishing relationships is progressively to immunize the animal and note the widening range of the precipitation. In support of this idea he gave the data tabulated on page 221.

Similar results were secured with other members of the grass family. However, in carrying his treatments as long as 245 days, in no case was a reaction secured with a non-grass.

MEZ and GOLKE (**24**) were the first to make a serious attempt to study plant relationships by means of serological studies. While the principal portion of their work was with the conglutinin method, they also used the precipitin reaction. They worked with a great number of angiosperms, and grouped them according to the relationships exhibited in these tests. Since that time MEZ and the workers in his laboratory have carried on considerable experimental work in their study of the so-called natural relationships of plants. Little of this work has touched upon legumes. RIVES (**28**) suggested the

TREATMENT OF ANIMAL (DAYS)	POSITIVE REACTION WITH	NEGATIVE REACTION WITH
	ANTIWHEAT SERUM	
19.....	Triticum, Hordeum, Secale	Zea
29.....	All the above plus Zea	Lolium, Bromus
36.....	All the above plus Lolium and Bromus	Oryza
146.....	All the above plus Oryza	.....
	ANTIMAIZE SERUM	
7.....	Zea, Euchlaena	Panicum, Triticum, and Hordeum
28.....	All the above plus Panicum	.....
50.....	All the above plus Triticum and Hordeum	Phalaris, Festuca, Bromus, and Lolium
109.....	All the above plus Phalaris, Festuca, Bromus, and Lolium	Avena
130.....	All the above plus Avena	.....

use of the precipitin method to measure the relationships existing between grapes. He found that grape hybrids which showed close relationships with the precipitin reaction could be grafted upon each other. Those which did not show close relationships by the precipitin reaction could not be grafted together.

The early workers in this field used crude extracts of the plant material, usually physiological salt solution extracts. GASIS (**12**) and WELLS (**33**) were the first to use purified plant proteins in immunological work. GASIS used the precipitin reaction and was successful in separating peas and beans, as well as several other plants. WELLS used purified zein and gliadin and was able to demonstrate that the anaphylactic reaction was equally as specific

and as severe with vegetable antigens as with animal proteins. WELLS and his associates followed up his early work with considerable additional experimentation (**20, 34–38, 40**). This work was largely with the anaphylactic reaction, although the other reactions were also used. Carefully prepared and purified plant proteins were used in each case. The following facts of particular interest in this work were demonstrated. (1) The specificity of the biological reactions is dependent upon the chemical constitution of the antigen and not upon its origin. Thus the use of the biological method in the determination of plant relationships is a measure of protein similarity. (2) Natural sensitization of guinea pigs may occur by feeding of the antigen. (3) Animals may be sensitized to two or more proteins at the same time, and after intoxication with one of the proteins, providing death does not occur, a reaction may be obtained by intoxication with the other.

The results of the previous work with legumes are presented in table I. This table shows that all the work, extending over a considerable period, done with a variety of serological methods, and in most cases unhampered by the influence of previous results, is in substantial agreement. Practically all of the work indicates that pea, vetch, lentil, and horse bean are more or less closely related and form one group. Among the beans *Phaseolus vulgarus*, *P. multiflorus*, and *P. nanus* have been grouped together. *P. raditus*, however, has been shown to possess a different protein complex from that of *P. vulgarus*. Red and white clover have been grouped together, with alfalfa closely related but still separate. Seven species of *Acacia* have been grouped together and separated from pea, vetch, and bean. Soy bean has been separated from all others. Ground nut (*Apios tuberosa*) has been shown to be different from either the pea or bean group. Lupine did not react with pea and lentil. Vignin, the purified globulin from cowpea, while reacting to a certain extent with the globulins of the other legumes, still appears to be different from any of the others used, that is, pea, vetch, lentil, soy bean, and horse bean.

While this work covers only a very small proportion of the legumes in which one is interested from the standpoint of cross

TABLE I

PREVIOUS WORK ON BIOLOGICAL SEPARATION OF LEGUMES

WORKER	DATE	REFERENCE	METHOD	RESULTS
Bertarelli	1903	5	Precipitin	Pea, bean, lentil, vetch, and horse bean, all separate
Magnus and Friedenthal	1907	23	Precipitin	Pea and vetch together; lupine separate group
Gasis	1908	12	Precipitin	Pea and lentil together; Phaseolus vulgarus and P. multiflorus nearly alike
Raubitschek	1909	27	Precipitin, anaphylaxis	Pea and lentil together; bean separate group
Azuma	1910	1, 2	Precipitin, anaphylaxis	Soy bean and horse bean different
Inomato	1910	14	Anaphylaxis	Different varieties of lentil alike
Ballner	1910	3	Complement fixation	Pea and lentil separate
Karawasa	1910	16	Anaphylaxis	Pea, lentil, and "grosse" bean different from bean
Chapman	1910	6	Precipitin	Seven strains of Acacia together Pea more closely related to Acacia than bean or vetch
Relander	1911	30	Precipitin	Red clover and vetch different; separates strains of red clover
Ballner and Burrows	1911	4	Complement fixation	Pea, vetch, and lentil together Phaseolus nanus and P. multiflorus together
Wendelstadt and Fellmer	1911	39	Precipitin, anaphylaxis, complement fixation	Pea, vetch, lentil, and horse bean together } Ground nut different from White and turkish bean together } either
Sauli	1911	31	Conglutinin	Pea, vetch, and broad bean together } Also separated strains in horse Red and white clover together } bean and red clover Alfalfa close to clovers but different
Golke	1915	13	Conglutinin, precipitin	All legumes together; pea and vetch nearest to lentil
Koketsu	1917	18	Precipitin	All legumes together; none close to horse bean
Kohz	1923	17	Conglutinin, precipitin	All legumes together
Ishiwora	1923	15	Abderhalden	Pea and soy bean separate
Wells et al*	1911	34	Anaphylaxis	Soy bean separate from others
	1913	35		Cowpea indistinct but separate
	1914	36		Pea, vetch, lentil, and horse bean togethe.
	1915	37		Kidney bean and adzuki bean separate from each other
	1916	38		

* Worked with purified proteins.

inoculation, the protein groups in every case agree with the established cross inoculation groups.

Experimentation

Previous work in this field has established the value of serological methods for classifying plant species into groups, whose members are more or less closely related physiologically; also that all groups which have been made of the Leguminosae by these methods agree with the cross inoculation groups of the root nodule bacteria. With these facts in mind, it was arranged to investigate the protein relationships existing between other members of the Leguminosae, including all the commonly cultivated legumes. The plants studied are grouped on the opposite page.

The seeds of the plants were selected for study, since practically all of the previous work had been done with seeds, and also since these represent the most readily available sources of material. Since the purpose was to establish relationships between species, rather than to detect differences between closely related forms, the methods of procedure were chosen with that in mind. Due both to their suitability and to their ease of manipulation, the precipitin and anaphylaxis reactions were chosen for this work. The use of the DALE (8) uterus strip method of anaphylaxis provides an additional advantage, in that a permanent visual record of the course of the reaction is secured.

Earlier workers with naturally occurring protein mixtures of either plant or animal origin, such as MAGNUS (21) and others, have shown that the length of duration of the immunization process influences to a considerable degree the breadth of the relationships exhibited by the antiserum. Serum produced by weak immunization and of low titre is of particular value in differentiating between closely allied forms (RELANDER 30). On the other hand, severe and long continued immunization tends to produce a serum reacting with a wide range of related species; GOHLKE (13) found all the legumes rather closely related. In this work it was attempted to secure moderate immunization with the production of sera which would be useful in the determination of plant relationships.

Since the purpose of this work was to study the physiological relationships, in so far as protein characteristics are concerned, it

was felt that an extract of the seeds which would contain some of all the proteins present would be more suitable for use as an antigen than a solution of the purified plant proteins. After a review of the

Alfalfa group	1. Alfalfa, *Medicago sativa*
	2. Hubam clover
	3. White sweet clover, *Melilotus alba*
	4. Fenugreek, *Trigonella foenumgraecum*
Clover	5. Red clover, *Trifolium pratense*
	6. White clover, *T. repens*
	7. Alsike clover, *T. hybridum*
Pea group	8. Garden pea, *Pisum sativum*
	9. Canada field pea, *P. sativum arvense*
	10. Broad or horse bean, *Vicia faba*
	11. Hairy vetch, *V. villosa*
	12. Spring vetch, *V. sativa*
	13. Wild vetch, *V. angustifolia*
Cowpea group	14. Cowpea, *Vigna sinensis*
	15. Lima bean, *Phaseolus limensis*
	16. Japan clover, *Lespedeza striata*
	17. Velvet bean, *Mucuna utilis*
	18. Beggarweed, *Desmodium tortuosum*
	19. Furze ?, *Ulex europaeus*
	20. Scotch broom ?, *Cytisus scoparius*
Garden bean group	21. Garden bean, *Phaseolus vulgarus*
Soy bean group	22. Soy bean, *Soja max*
Lupine group	23. Blue lupine, *Lupinus angustifolius*
	24. Yellow lupine, *L. luteus*
	25. White lupine, *L. alba*
	26. Serradella, *Ornithopus sativus*
Sanfoin group	27. Sanfoin, *Onobrychis sativa*
	28. Dalea,* *Dalea alopecuroides*
	29. Kidney vetch,* *Anthyllis vulneraria*

* Dalea and kidney vetch have not been tested against each other for cross inoculation. Neither crosses with any of the other in the list.

literature and several preliminary trials, a 2 per cent sodium chloride solution was selected as the extracting agent. The seeds were ground to a fine meal and extracted with the saline solution in the proportion of 1 part of meal to 10 of solution. The extractions were continued for 30 minutes at room temperature, and then filtered

clear through a mat of paper pulp. The clear filtrate was used as the antigen, and fresh extracts were prepared each time the material was used.

PRECIPITIN REACTION

Antisera were prepared for the saline extracts of the seeds of spring vetch, garden pea, alsike clover, white clover, garden bean, lima bean, Japan clover, yellow lupine, serradella, sanfoin, soy bean, kidney vetch, fenugreek, alfalfa, and dalea. Rabbits were used in the preparation of the antisera. The injections were made intraperitoneally. With the exception of the kidney vetch, nine injections were made, comprising a total of 45 cc. of antigen in each case. With kidney vetch ten injections and 50 cc. of antigen were used. In every case the rabbits remained in good condition and steadily gained in weight.

Preliminary tests were carried out to determine the value of removing the albumen in extracts by heat coagulation. Extracts were heated to 60° C. for 30 minutes and the coagulum filtered off. No significant difference between heated and unheated extracts was noted, and the unheated extracts were used in all the tests reported here.

The animals were aseptically bled from the heart and the serum collected and stored under sterile conditions in the ice box. In cases where there was any doubt as to the presence of bacteria in the sera, phenol was added to make a 0.5 per cent concentration.

For the precipitin test, the antigen was diluted in concentrations ranging from 1–100 to 1–51,200. To 1 cc. of the diluted antigen in a 9 mm. test tube, 0.1 cc. of undiluted serum was added. The two solutions were thoroughly mixed and incubated at 37° C. for 12 hours. Care was taken to keep the materials free from contamination and no trouble was experienced from bacterial growth in the tubes during the incubation period. Controls were carried out in every case. Each antiserum was tested against antigens prepared from twenty-nine different legumes, comprising members from at least nine different cross inoculation groups. Tables II and III record the results of these tests. Table II gives as an illustration the complete data which were secured with the spring vetch antiserum. Table III summarizes the data from all the experiments.

TABLE II

ACCORDING TO PRECIPITIN REACTION WITH THEIR SEED PROTEINS

TABLE II

GROUPING OF LEGUMES ACCORDING TO PRECIPITIN REACTION WITH THEIR SEED PROTEINS
SPRING VETCH ANTISERUM

CROSS INOCULATION GROUP	ANTIGENS FROM SEEDS OF	DILUTION OF ANTIGEN									
		200	400	800	1600	3200	6400	12800	25600	51200	Control
Alfalfa	Alfalfa	+	+	−	−	−	−	−	−	−	−
	White sweet clover	++	+	−	−	−	−	−	−	−	−
	Hubam clover	+	−	−	−	−	−	−	−	−	−
	Fenugreek	++	+	−	−	−	−	−	−	−	−
Clover	Red clover	+	+	+	−	−	−	−	−	−	−
	White clover	++	+	−	−	−	−	−	−	−	−
	Alsike clover	+	−	−	−	−	−	−	−	−	−
Garden pea	Garden pea	+++	+++	++	+	+	+	−	−	−	−
	Canada field pea	+++	+++	++	++	+	+	−	−	−	−
	Broad bean	++	++	+	+	+	+	−	−	−	−
	Hairy vetch	+++	+++	++	+	+	+	−	−	−	−
	Spring vetch	+++	+++	++	++	+	+	+	−	−	−
	Wild vetch	+++	+++	++	++	+	+	+	−	−	−
Cowpea	Cowpea	+	+	−	−	−	−	−	−	−	−
	Lima bean	++	+	−	−	−	−	−	−	−	−
	Japan clover	+	−	−	−	−	−	−	−	−	−
	Velvet bean	++	+	+	+	−	−	−	−	−	−
	Beggarweed	++	+	+	+	−	−	−	−	−	−
	Scotch broom	++	++	+	−	−	−	−	−	−	−
	Furze	++	++	+	−	−	−	−	−	−	−
Garden bean	Garden bean	++	+	+	−	−	−	−	−	−	−
Soy bean	Soy bean	+	+	−	−	−	−	−	−	−	−
Lupine	Yellow lupine	++	++	+	+	−	−	−	−	−	−
	Blue lupine	++	++	+	−	−	−	−	−	−	−
	White lupine	++	++	+	−	−	−	−	−	−	−
	Serradella	++	+	+	+	−	−	−	−	−	−
Sanfoin	Sanfoin	++	+	+	−	−	−	−	−	−	−
Dalea	Dalea	+	−	−	−	−	−	−	−	−	−
Kidney vetch	Kidney vetch	+	+	−	−	−	−	−	−	−	−

TABLE III

GROUPING OF LEGUMES ACCORDING TO PRECIPITIN REACTION WITH SEED PROTEINS (HIGHEST DILUTION OF ANTIGEN WHICH GAVE PRECIPITATION IS LISTED; MOST OF RESULTS AT DILUTIONS OF 1600 AND ALL BELOW THAT DILUTION ARE OMITTED)

ANTIGENS FROM SEED OF	ALFALFA	FENUGREEK	ALSIKE CLOVER	WHITE CLOVER	SPRING VETCH	GARDEN PEA	LIMA BEAN	JAPAN CLOVER	GARDEN BEAN	SOY BEAN	YELLOW LUPINE	SERRADELLA	SANFOIN	DALEA	KIDNEY VETCH
Alfalfa	12800	6400	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
White sweet clover	12800	6400	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Hubam clover	12800	6400	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Fenugreek	6400	12800	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Red clover	.....	.....	6400	6400	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
White clover	.....	.....	6400	6400	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Alsike clover	.....	.....	6400	6400	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Garden pea	.....	.....	.....	.....	6400	12800	.....	.....	.....	.....	.....	.....	.....	.....	.....
Canada field pea	.....	.....	.....	.....	6400	12800	.....	.....	.....	.....	.....	.....	.....	.....	.....
Broad bean	.....	.....	.....	.....	6400	6400	.....	.....	.....	.....	.....	.....	.....	.....	.....
Hairy vetch	.....	.....	.....	.....	6400	12800	.....	.....	.....	.....	.....	.....	.....	.....	.....
Spring vetch	.....	.....	.....	.....	12800	12800	.....	.....	.....	.....	.....	.....	.....	.....	.....
Wild vetch	.....	.....	.....	.....	12800	12800	.....	.....	.....	.....	.....	.....	.....	.....	.....
Cowpea	.....	.....	.....	.....	.....	.....	3200	1600	.....	.....	.....	.....	.....	.....	.....
Lima bean	.....	.....	.....	.....	.....	.....	12800	3200	.....	.....	.....	.....	.....	.....	.....
Japan clover	.....	.....	.....	.....	.....	.....	1600	6400	.....	.....	.....	.....	.....	.....	.....
Velvet bean	.....	.....	.....	.....	.....	.....	3200	3200	.....	.....	.....	.....	.....	3200	.....
Beggarweed	.....	.....	.....	.....	.....	.....	3200	3200	.....	.....	1600	3200	1600	3200	.....
Scotch broom	.....	.....	.....	.....	.....	.....	3200	6400	.....	.....	3200	1600	1600	.....	3200
Furze	.....	.....	.....	.....	.....	.....	3200	6400	.....	.....	1600	1600	.....	3200	3200
Garden bean	.....	.....	.....	.....	.....	.....	.....	.....	25600	.....	.....	.....	.....	.....	.....
Soy bean	.....	.....	.....	.....	.....	.....	.....	.....	.....	12800	.....	.....	.....	.....	.....
Yellow lupine	.....	.....	.....	.....	.....	.....	.....	1600	.....	.....	6400	6400	3200	.....	.....
Blue lupine	.....	.....	.....	.....	.....	.....	.....	1600	.....	.....	6400	6400	3200	.....	.....
White lupine	.....	.....	.....	.....	.....	.....	.....	1600	.....	.....	6400	6400	1600	.....	.....
Serradella	.....	.....	.....	.....	.....	.....	.....	1600	.....	.....	6400	12800	3200	.....	.....
Sanfoin	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1600	1600	12800	.....	.....
Dalea	.....	.....	.....	.....	.....	.....	.....	1600	.....	.....	.....	.....	.....	12800	3200
Kidney vetch	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3200	6400

Spring vetch and garden pea antisera react much alike; in each case garden pea, Canada field pea, broad bean, hairy vetch, spring vetch, and wild vetch are placed in one group which is quite distinct from all other legumes tested. It will be noted that these legumes also belong to a single cross inoculation group. Most of them have been used by previous workers and similar results secured.

The alsike and white clover antisera appear to be almost identical. In each case red, white, and alsike clover are placed together in one group, considerably separated from any of the other legumes tested. This group likewise corresponds to the cross inoculation group, and also checks with the previous work which has been done with these plants.

With sanfoin antiserum the homologous antigen reacted in a dilution of 1–12,800; yellow lupine, blue lupine, and serradella proteins reacted in a dilution of 1–3200; and velvet bean, beggarweed, and white lupine protein reacted in a dilution of 1–1600. As measured by the cross inoculation tests sanfoin belongs by itself, and the precipitin tests might readily be interpreted to substantiate this. According to the precipitin test, the members of the lupine cross inoculation group are closely related; also velvet bean and beggarweed appear somewhat closely related.

The antisera prepared for yellow lupine and serradella react much alike; yellow, blue, and white lupine, and serradella are grouped together by this test as well as by the cross inoculation tests. Beggarweed, Scotch broom, furze, and sanfoin also appear to be somewhat closely related. Japan clover and lima bean antisera both group cowpea, lima bean, Japan clover, velvet bean, beggarweed, Scotch broom, and furze together, and these belong to a single cross inoculation group. The grouping is not nearly so definite as has been seen in certain other cases; in fact, members of the lupine group and dalea appear as closely related to Japan clover as does cowpea. This cross inoculation group represents a wide range of species, from the standpoint of botanical classification, and it may be regarded as remarkable that this close relationship is exhibited in regard to protein characteristics.

With garden bean antiserum the homologous protein reacted in a

dilution of 1–25,600, while the highest reaction dilution of any heterologous protein was 1–1600. This separation checks the results of other workers, and also corresponds to the cross inoculation group. The tests with soy bean antiserum place soy bean in a class by itself. The homologous protein reacted in a dilution of 1–12,800, while the highest reacting dilution of any heterologous antigen was 1–1600. This checks with the results of previous work and also with the cross inoculation group.

With kidney vetch antisera the homologous antigen reacted in a dilution of 1–6400. Scotch broom, furze, and dalea proteins reacted in dilutions of 1–3200. With the possible exception of dalea, which has not yet been tested, kidney vetch root nodule bacteria do not inoculate any of the legumes used in this test. The antisera which were prepared for fenugreek and alfalfa react much alike. Alfalfa, white sweet clover, Hubam clover, and fenugreek are placed in a group somewhat well separated from others. This corresponds to the cross inoculation tests.

Dalea antiserum gave a positive reaction with the homologous antigen in a dilution of 1–12,800. Velvet bean, beggarweed, furze, and kidney vetch proteins each reacted in a dilution of 1–3200. Dalea belongs to a cross inoculation group different from any of the legumes used in this test, with the possible exception of kidney vetch.

Anaphylaxis reaction

As a check upon the results of the precipitin test, many of the proteins were used in anaphylaxis tests. Two methods were used in these experiments, the ordinary gross anaphylaxis one and the uterus strip method of Dale.

In the gross anaphylaxis experiments, guinea pigs of 250–400 gm. weight were injected intraperitoneally with the previously described saline extracts of the legume seeds. After a suitable period of incubation the animals were given intoxicating doses of the various proteins by intraperitoneal injection.

In the uterus strip method of anaphylaxis the technique of Dale (**7, 8**) was used. The animals were sensitized as in the gross anaphylactic tests. At the end of the incubation period they were killed by a sharp blow on the head, bled by cutting the throat and the two horns of the uterus immediately removed. These were sus-

pended separately in oxygenated baths containing a modified Locke-Ringer solution[2] which were held at 37°–38° C. by a surrounding water bath. One end of the uterus strip was fastened to a rigid support at the bottom of the bath and the other was fastened to a thread, which passed over a series of pulleys and in turn was fastened to a lever. The end of the lever held a needle point which was made to impinge upon the surface of a smoked paper upon the revolving drum of a kymograph. After a relatively short time (20–30 minutes) the uterus strip assumed a regular rhythmic contraction and relaxation. When this occurred one of the protein extracts was added to the Locke-Ringer bath; 1 cc. of protein extract in 50 cc. of Locke-Ringer solution. The addition of the homologous protein causes a very decided contraction of the muscle, persisting over a considerable period of time and inhibiting the regular rhythmic contraction and relaxation. The addition of a heterologous protein causes no such reaction in the tissue.

A piece of tissue which has been saturated with the homologous antigen will not give another reaction upon subsequent additions of the homologous antigen. The reaction of the tissue in the anaphylaxis test is different in this respect from the general reaction of such tissue to poisons and excitatory agents. In this way it was possible to test out various protein extracts against the sensitized uterus strip and thus obtain a permanent visible record of the course of the reaction.

Gross anaphylaxis tests

Table IV records the data of two such experiments. In one case the guinea pigs were sensitized with red clover and in the other with kidney vetch. In the red clover set nothing is shown other than the fact that pea is not closely related to red clover in its protein characteristics. In the kidney vetch set, furze is shown to be closely related to kidney vetch, dalea slightly related, and serradella much more closely. The data in both cases support the results of the precipitin tests.

Table V records the results of the cowpea series. Japan clover and lima bean are shown to be rather closely related to cowpea,

[2] Modified Locke-Ringer solution was made up as follows: sodium chloride 2.00 gm., potassium chloride 0.42 gm., calcium chloride 0.12 gm., dextrose 1.00 gm., sodium bicarbonate 0.50 gm., water, glass redistilled, 1000 cc.

TABLE IV

GROUPING OF LEGUMES ACCORDING TO ANAPHYLAXIS REACTION WITH THEIR SEED PROTEINS

GUINEA PIG NO.	SENSITIZATION		INCUBATION (DAYS)	INTOXICATION (DOSE 2 CC.)		SECOND INTOXICATION, AFTER 24 HOURS (DOSE 2 CC.)		PROTECTION
	Protein	Dose (cc.)		Protein	Reaction	Protein	Reaction	
21	Red clover	1 of 1–100	21	Pea	None	Red clover	Slight	Partial (?)
22	Red clover	1 of 1–100	21	Red clover	Moderate	Red clover	None	Complete
23	Kidney vetch	1	14	Dalea	Slight	Kidney vetch	Mild	Partial
24	Kidney vetch	1	14	Dalea	Slight	Kidney vetch	Mild	Partial
25	Kidney vetch	1	14	Furze	Severe, dead			
26	Kidney vetch	1	14	Furze	Severe, dead	Kidney vetch	None	Complete
27	Kidney vetch	1	14	Serradella	Very mild	Kidney vetch	Moderate, severe	None
28	Kidney vetch	1	14	Serradella	Very mild	Kidney vetch	Moderate, severe	None
29	Kidney vetch	1	14	Kidney vetch	Severe	Kidney vetch	None	Complete
30	Kidney vetch	1	14	Kidney vetch	Severe	Kidney vetch	None	Complete

TABLE V

GROUPING OF LEGUMES ACCORDING TO GROSS ANAPHYLAXIS REACTION WITH SEED PROTEINS

GUINEA PIG NO.	SENSITIZATION WITH COWPEA PROTEIN (DOSE 1 CC. OF 1–10)	INCUBATION PERIOD (DAYS)	INTOXICATION (DOSE 2 CC.)		SECOND INTOXICATION AFTER 24 HOURS, WITH COWPEA PROTEIN (DOSE 2 CC.)	PROTECTION
			Protein	Reaction	Reaction	
1		29	Cowpea	Severe, dead		
2		29	Cowpea	Severe	None	Complete
3		29	Japan clover	Moderately severe	Very mild	Partial
4		29	Japan clover	Moderately severe	Very mild	Partial
5		29	Lima bean	Mild	Mild	Partial
6		29	Lima bean	Mild	Very mild	Partial
7		29	Garden bean	None	Severe	None
8		29	Garden bean	None	Severe	None
9		30	White sweet clover	Very mild	Severe	None
10		30	White sweet clover	Very mild	Severe	None
11		30	White clover	Very mild	Moderate	None
12		30	White clover	Very mild	Moderately severe	None
13		30	Garden pea	Very mild	Severe	None
14		30	Garden pea	Very mild	Severe, dead	None
15		30	Cowpea	Moderate	None	Complete

while garden bean, white sweet clover, white clover, and garden pea bear very little relation to cowpea. This again checks the results of the precipitin tests.

UTERUS STRIP ANAPHYLAXIS TESTS

While the uterus strip method of measuring and recording anaphylactic shock represents an ideal method in many respects, care must be used in the interpretations of results when working with such natural mixtures of proteins as occur in the legume seeds. The degree of sensitization determines to a considerable extent the degree of relationship which will be shown. For example, guinea pig no. 27 (table VI) was so heavily sensitized with lima bean protein, 2 cc. injected, that the uterus strip reacted with sanfoin and yellow lupine proteins practically as strongly as it did with lima bean protein. Previous tests had not indicated any close relationship between these legumes. On the other hand, guinea pig no. C99.3 (table VI) also sensitized with lima bean protein, but with only 2 cc. of a 1–10 dilution, was not sensitized heavily enough to give a reaction with either cowpea or Japan clover proteins, although it did react with lima bean protein. Guinea pig no. 55 (table VI and fig. 1 *b*), which received 0.5 cc. of lima bean protein, reacted with both cowpea and Japan clover proteins as well as lima bean protein, and this checks with both the precipitin and the cross inoculation tests. Tables VI and VII give the results of these tests and figs. 1 and 2 show photographs of a few of the charts. In the main these results check closely with the results of the precipitin and gross anaphylaxis tests. Due to individual variations in the degree of sensitization, however, groups in some cases might be enlarged and in other cases made smaller, if based entirely on the results of this test.

With guinea pig no. 17 the close relationship between broad bean, garden pea, and spring vetch is indicated. In fig. 2 the charts *g* and *h* produced with this pig are reproduced. The pig was sensitized with broad bean antigen and the addition of cowpea and lima bean antigens produced no reaction, while the addition of spring vetch antigen caused a marked contraction of the uterus strip with a temporary inhibition of the rhythmic contractions and relaxations. This addition of spring vetch antigen was enough to saturate the

TABLE VI

GROUPING OF LEGUMES ACCORDING TO THE ANAPHYLAXIS REACTION WITH SEED PROTEINS (UTERUS STRIP METHOD); INCUBATED 14 DAYS

GUINEA PIG NO.	SENSITIZATION		INTOXICATION					
	Protein	Dose	First	Second	Third	Fourth	Fifth	Sixth
17.....	Broad bean	2 of 1–10	NaCl −	Garden bean +	Cowpea −	Garden pea +++	.....	.....
17.....	Broad bean	2 of 1–10	Cowpea −	Lima bean −	Spring vetch +++	Spring vetch −	Broad bean −	.....
29.....	Cowpea	0.5	Garden pea −	Furze ++	Cowpea −	.....	.....	.....
29.....	Cowpea	0.5	Garden pea −	Furze ++	Cowpea −	.....	.....	.....
27.....	Lima bean	2.0	Sanfoin +	Yellow lupine +	Lima bean ++	.....	.....	.....
55.....	Lima bean	0.5	Cowpea +++	Cowpea +	Sanfoin −	Lima bean −	.....	.....
C 99.3...	Lima bean	2 of 1–10	Japan clover −	Cowpea −	Lima bean ++	.....	.....	.....
C103.1...	Lima bean	2 of 1–10	Garden bean ±	Cowpea −	Lima bean +++	Lima bean −	.....	.....
C 86.2...	Japan clover	2 of 1–10	Garden pea −	Japan clover −−−	.....	.....	.....	.....
34.....	Beggarweed	0.5	Garden bean −	Broad bean −	Lima bean −	Scotch broom −	Beggarweed ++	.....
34.....	Beggarweed	0.5	Garden pea −	Velvet bean −	Cowpea −	Scotch broom −	Beggarweed ++	.....
13.....	Furze	0.5	Blue lupine −	Sanfoin −	White sweet clover −	Scotch broom ++	Furze +++	.....
13.....	Furze	0.5	Garden pea −	White clover −	Cowpea −	Scotch broom ±	Furze +++	.....
5.....	Scotch broom	0.5	Japan clover ±	Japan clover −	Serradella −	Dalea −	Kidney vetch ++	Furze +++
5.....	Scotch broom	0.5	Beggarweed ++	Beggarweed −	Sanfoin +	Sanfoin −	Furze +++	.....
6.....	Scotch broom	1.0	Sanfoin −	Velvet bean −	Yellow lupine −	Furze −−	.....	.....
12.....	Yellow lupine	2 of 1–10	Serradella −	Sanfoin +	Sanfoin +	Blue lupine +	Yellow lupine ±	.....
21.....	Yellow lupine	2 of 1–10	Sanfoin +++	Yellow lupine −	.....	.....	.....	.....
35.....	Serradella	0.5	Kidney vetch −	Soy bean −	Blue lupine ++	Serradella −	.....	.....
38.....	Serradella	0.5	Spring vetch −	Soy bean +	Yellow lupine ++	Yellow lupine −	Serradella ++	.....
38.....	Serradella	0.5	Garden pea ±	Kidney vetch ±	Sanfoin +	Sanfoin ±	Serradella −	.....

Reaction: −, none; ±, doubtful; +, weak; ++, medium; +++, strong.

TABLE VII

GROUPING OF LEGUMES ACCORDING TO ANAPHYLAXIS REACTION WITH SEED PROTEINS (UTERUS STRIP METHOD); INCUBATED 14 DAYS

GUINEA PIG NO.	SENSITIZATION		INTOXICATION					
	Protein	Dose	First	Second	Third	Fourth	Fifth	Sixth
45	Kidney vetch	1.0	Serradella ±	Beggarweed −	Furze +++	Furze +	Kidney vetch −	.....
45		1.0	Hairy vetch ±	White clover −	Dalea −	Furze +++	Kidney vetch −	.....
C116.3		0.5	White sweet clover +++	Beggarweed +++	Serradella −	Kidney vetch +++	.....	.....
C116.3		0.5	White sweet clover +++	Velvet bean +++	Dalea −	Kidney vetch +++	.....	.....
19	Dalea	0.25	Kidney vetch ++	Kidney vetch +	Yellow lupine −	Japan clover −	Dalea −	.....
19		0.25	Furze +++	Furze +	Beggarweed −	Dalea −	.....	.....
24		1.0	White sweet clover ±	Furze +++	Dalea −	.....	.....	.....
24		1.0	White clover −	Beggarweed −	Japan clover −	Furze +++	Dalea −	.....
33	White sweet clover	0.5	Velvet bean −	Funugreek −	White sweet clover ++	.....	.....	.....
33		0.5	Scotch broom −	Hubam clover −	White sweet clover +	.....	.....	.....
41		0.5	Yellow lupine +	White sweet clover ++	White sweet clover +	.....	.....	.....
41		0.5	Garden pea +	Hubam clover +	White sweet clover +	.....	.....	.....
52		0.5	Beggarweed ++	Beggarweed −	Kidney vetch +++	Alsike clover −	Alfalfa −	White sweet clover −
52		0.5	Dalea −	Scotch broom −	Kidney vetch ++	White clover −	Hubam clover	White sweet clover +

Reaction: −, none; ±, doubtful; +, weak; ++, medium; +++, strong.

tissue, since subsequent additions of both spring vetch and broad bean antigens failed to produce any further reaction. With the other horn of the uterus from this pig, the addition of 1 cc. of a 2 per cent

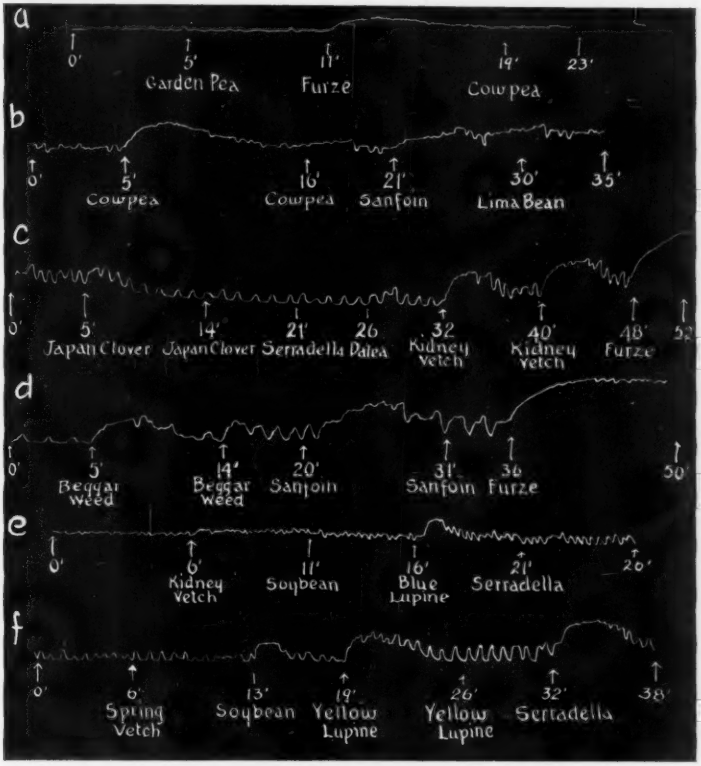

FIG. 1.—Reaction to various protein solutions of uterus strips from guinea pigs sensitized with seed protein from: *a*, cowpea (guinea pig no. 29); *b*, lima bean (no. 55); *c*, *d*, Scotch broom (no. 5); *e*, serradella (no. 35); *f*, serradella (no. 38).

salt solution and of cowpea antigen failed to produce any reaction. The addition of garden bean antigen produced a very slight interruption of the rhythmic contractions and relaxations, while the addition of garden pea antigen produced a pronounced contraction of the uterus strip.

In the reactions of cowpea and lima bean pigs (fig. 1 *a*, *b*) evidence was found which justifies placing cowpea, lima bean, and furze in one group. Furze and Scotch broom (fig. 1 *c*, *d*) are shown to

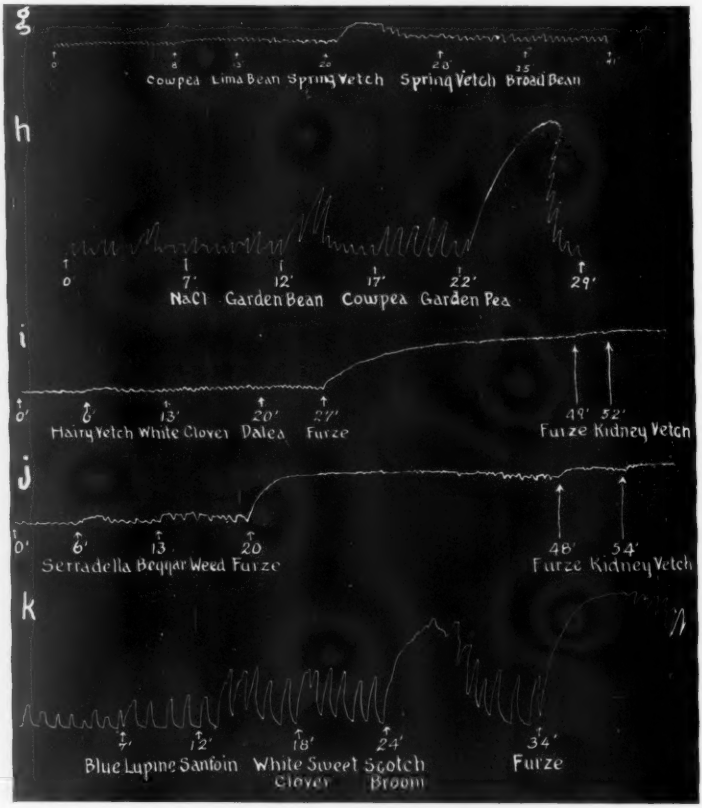

FIG. 2.—Reaction to various protein solutions of uterus strips from guinea pigs sensitized with seed protein from: *g*, *h*, broad bean (guinea pig no. 17); *i*, *j*, kidney vetch (no. 45); *k*, furze (no. 13).

be closely related to each other and to be more distantly related to Japan clover, dalea, kidney vetch, beggarweed, and sanfoin. The chart for guinea pig no. 13 (fig. 2 *k*) gives an example of a case in which a pronounced reaction was secured by the addition of

a heterologous antigen, without giving a saturation effect for the homologous antigen. The pig was sensitized with furze antigen, and the addition of blue lupine, sanfoin, and white sweet clover antigens to the tissue produced no reaction. The addition of Scotch broom antigen gave a decided contraction, but without complete saturation of the tissue for the homologous antigen, since a subsequent addition of furze antigen produced very strong contraction of the tissue. This reaction indicates that while Scotch broom and furze proteins are closely related they are not identical.

The reactions of the yellow lupine and serradella pigs gave ample evidence for the grouping together of the lupines and serradella; however it is also indicated that sanfoin is closely related. Guinea pig no. 45 (fig. 2 *i*, *j*) indicates the close relationship between kidney vetch and furze, which corresponds with other data which have been secured. The reactions of guinea pig no. C116.3 (table VII) would indicate that hairy vetch, beggarweed, white sweet clover, and velvet bean are each closely related to kidney vetch, and this is not in accordance with other data. The reactions secured with the dalea pigs (table VII) indicate a close relationship between kidney vetch, furze, and dalea, which is in accordance with the data of the precipitin tests. Rather erratic results were secured with the pigs sensitized to white sweet clover (table VII). Frequently extracts of distantly related legumes, as judged by the botanical and precipitin classifications, reacted much more vigorously than did sweet clover itself. With such a wide variety of results, it would hardly be safe to draw any conclusions relative to the legumes most closely associated with white sweet clover.

In general, the results of this test are in harmony with the results of the precipitin and gross anaphylaxis tests.

Agglutination of nodule bacteria with legume protein antisera

SIMON (**32**) suggested that there might be a relation between the proteins of the legumes and the nodule bacteria, which could be determined by serological methods. No data are reported to substantiate the idea, and there has been no further reference to the subject found in the literature. If the theory of SIMON is correct, one might expect the legume seed antisera to agglutinate the nodule

bacteria from the same species. This was tested and the results indicated that the protein of the nodule bacteria and the legume seed are not closely allied.

Summary

1. A consideration of the factors which might be responsible for the adaptation of certain strains of the legume nodule bacteria to certain species of legumes and not to others suggests that the chemical complex of the host plants may be responsible. It seems reasonable to believe that any two plants, each of which is inoculated with the same strain of root nodule bacteria, must be more or less closely related physiologically. The physiology of any plant comprises a great number of different factors, any one of which might be the controlling one in the matter of inoculation by a specific organism.

2. Similar relationships between the physiological complex of plants and their resistance or non-resistance to invasion and the production of disease have been noted in the case of plant parasites.

3. Additional evidence of the fact that the members of a cross inoculation group in the legumes may be physiologically related is offered by the fact that the cowpea root nodule organism and *Bact. vignae*, the cowpea leaf spot organism, infect exactly the same group of plants.

4. The protein characteristics of plants offer one of the most easily measured factors in their chemistry. Serological methods of measuring the relationships existing between the protein characteristics of plants have been developed and used to a considerable extent. All such studies, which have previously been made of the protein characteristics of legumes, placed them in similar groups to the cross inoculation ones.

5. In the work reported in this paper, the protein characteristics of twenty-nine species of the commonly cultivated legumes have been studied by means of the precipitin and anaphylaxis reactions.

6. The results of these tests show that all members of any cross inoculation group are closely related with respect to the protein characteristics of their seeds, and in the majority of cases all legumes which possess closely related seed protein complexes cross inoculate. The latter point, however, is not true in all cases.

7. Several possibilities suggest themselves to account for these facts. (1) The protein characteristics of the host plant may be the controlling factors in determining whether a particular bacterial type is capable of causing inoculation, and a more careful and detailed analysis of the purified proteins from the seeds of the plant might demonstrate that relationship. (2) If the protein characteristics of the plant are the controlling factors, the protein characteristics of the root, and more definitely of the root hairs, must be the important point, since this is the point of attack by the bacteria. It is probable also that a somewhat different series of plant relationships might be established if the protein characters of the root hairs were taken as the subject for study. (3) It is entirely possible that the protein characters are not the controlling factors in the matter of inoculation by the root nodule bacteria, and that the protein relationships shown to exist between members of the cross inoculation groups are correlated to a certain extent with another physiological factor which is the controlling one.

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BEHAVIOR OF CERTAIN FERN PROTHALLIA UNDER PROLONGED CULTIVATION

DAVID M. MOTTIER

(WITH PLATE X AND SEVEN FIGURES)

At the Cincinnati meeting of the Botanical Society of America, in December 1923, the writer gave a preliminary report upon the behavior of the prothallia of *Matteuccia nodulosa* (Michx.) Fernald (*Onoclea Struthiopteris*), and *Osmunda claytoniana* L. under prolonged cultivation. The data presented at that time were based upon prothallia that were a little more than a year old in the case of *M. nodulosa*, and seven months old in that of *O. claytoniana*. A number of the same prothallia have been kept growing, so that the results of four years of continuous observations are now at hand, and the more important features of these will be set forth in the following paper.

Historical

The ordinary behavior of these prothallia in culture is so well known that a description of their development from the spore will not be repeated. In the literature one finds references to old prothallia of the Polypodiaceae and Osmundaceae, but the writer has not been able to find any record of continuous growth in culture from the time of sowing to the end of the fourth year. It is well known that the prothallia of the two genera under consideration, as well as those of other Polypodiaceae, when grown beyond the ordinary time for the production of sporophytes, may branch both dichotomously and by means of lateral proliferations. The proliferations may spring from both the upper and the under sides of the midrib (MOTTIER **9**), and from the margins of the wings of the gametophyte.

HOFMEISTER (**6**) calls attention to lateral proliferations or adventitious shoots in the prothallia of *Notochlaena*, *Allosurus*, and *Gymnogramma calomelanos*. Similar structures have been described later by KNY (**7**), GOEBEL (**3**), CAMPBELL (**1, 2**), and doubtless by many other observers. KNY, in referring to *Osmunda regalis*, states that the prothallia produce adventitious shoots in a high degree.

These arise from the margin of the wings and not from the surface. In a footnote, however, he mentions a single case in which a shoot sprang from the midrib on the under side, and upon this shoot numerous archegonia were developed. He regarded the shoot as an abnormality. GOEBEL (3) has called attention to the production of numerous and varied adventitious shoots from both young and older or mature tissues of the prothallia of *Gymnogramma leptophylla* Desv. In older prothallia shoots arise either from the edge or from the surface. In regard to the dichotomous branching of prothallia, the earliest account that the writer has found is that of GOEBEL (3), who described and figured this mode of branching in *Osmunda regalis*. The dichotomy, which occurs rarely, takes place by the regular bifurcation of the growing point. In speaking of the branching in *Osmunda*, CAMPBELL (1) states:

> Not infrequently, especially in *O. claytoniana*, the young prothallia branch irregularly, or in some cases there seems to be a true dichotomy (*l.c.* fig. 21); but in the latter case one of the branches finally grows faster than the other, which is suppressed, and the resultant prothallium does not differ much in appearance from the ordinary type.

CAMPBELL calls attention also to "secondary prothallia" in *Osmunda*, which may arise from the margin and from the lower surface. By the death of the older tissue these shoots become isolated, continue to grow, and produce normal sexual organs.

In regard to the continued growth of old prothallia that do not bear sporophytes, GOEBEL (3) refers to a much branched four-year old prothallium of *Osmunda regalis* that had attained a length of 3–4 cm., resembling in appearance the liverwort *Pellia epiphylla*. He states further that among these prothallia old Polypodiaceous prothallia were found exhibiting the same habit of growth. He did not mention the species referred to, and no data as to the method of cultivation were given. As will be shown in a following paragraph, prothallia of *O. claytoniana* may attain a length of 3.5 cm. in fourteen months.

Method of culture

Stock cultures of the prothallia were first made upon sterilized woods earth, and as soon as the plants had attained a width of 2–4 mm. across the wings, the most vigorous individuals were trans-

planted to separate vessels. In all cases, unglazed earthenware flower pot saucers 5 or 6 inches in diameter were used. Each culture was covered with a bell glass, which was usually propped up on one side to a height of about one-fourth of an inch to insure ventilation and to facilitate evaporation, should the earth be made too wet in watering. Cultures were always watered by sub-irrigation. A more detailed account of the method of culture has been published elsewhere (8). From twenty to twenty-five prothallia can be conveniently spaced in each saucer. Text figs. 1, 2, and 6 show the appearance of the transplanted prothallia from four to seven months old.

In order to grow prothallia for a long period of time, it is necessary of course to prevent the production of sporophytes, and to amputate, without injury to the prothallia, such sporophytes as may develop in spite of precautions. Since the plants were watered by sub-irrigation, sporophytes rarely appeared, especially in older plants. After a little practice a sporophyte possessing the first visible leaf may easily be amputated close to the surface of the prothallium with a sharp, lance-shaped needle or the blade of a safety razor. When the operation is successfully done, scarcely any traumatic effects result, and such plants continue to grow as do those upon which no sporophytes have developed. For reasons that will be mentioned later, sporophyte production is more easily prevented in old prothallia.

Prothallia of other species are more easily grown than those of the two selected, but under the greenhouse conditions available it was necessary to select species whose prothallia could always be distinguished from "weed" prothallia that almost invariably find their way into the cultures. Even in cultures grown on outside window shelves of the laboratory, far removed from other ferns, "weed" prothallia made their appearance, having developed from wind-blown spores. The writer has always been able, with the aid of a simple magnifier, to distinguish between the prothallia of *Matteuccia nodulosa* and *Osmunda claytoniana* and those of other ferns grown in the greenhouse, as well as those from other sources. Diffused light in both winter and summer proved most favorable for the growth of the plants, although direct sunlight for a short period in early morning did not seem to be unfavorable.

Growing fern prothallia for a short period is a comparatively easy task, since few foreign plants will tend to appear on sterilized soil, especially if the prothallia are crowded; but when cultivation is extended through months and years, many difficulties confront the experimenter. Chief among the trouble makers are moss protonema, which tend to form a mat on the surface of the soil, unicellular green algae, and certain soil-loving blue-green algae, as species of *Lyngbya*, and finally fungi and insect pests. Spores and dried parts of the plants mentioned are always in the air, and it is not possible to prevent their gaining access to the surface of the cultures.

Although mosses gain a foothold in the cultures after a time, they are easily held in check. The leafy stems and protonemal mats may be removed from time to time with needle and forceps. When such removal takes along a layer of soil it is necessary to add carefully new sterilized earth. Whenever moss and algal growth became too well established to be held in check by the foregoing method, the prothallia were carefully transplanted to a new dish of sterilized soil. A few times during the past four years it became easier to move the crop, so to speak, from the old to a new field than to try to clean out the weed plants. In some cultures frequent scratching or cultivation of the surface of the soil between the plants was very helpful.

Of the blue-green algae, species of *Lyngbya* were the most troublesome, both in summer and winter. In a few cultures, especially if the soil were permitted to become a little too moist, they tended to form a crust upon the soil, and to spread upon the surface of the prothallia as well. As a remedy the culture was permitted to become somewhat dry, after which the crust was removed and new earth added. *Protococcus* and other unicellular green algae gave little trouble further than to coat over the sides of the earthen saucers.

Of the animal pests, the larvae of a small, gnatlike Dipterous insect were the most troublesome. The small, maggot-like larvae burrow in the soil, feeding upon the young rhizoids and tender parts of the under surface of the thallus. Thus concealed they may do considerable damage before their presence is discovered. At times they come to the surface to devour the tender parts of the thallus above the ground. In crawling over the soil they leave a glistening trail not unlike that of a tiny slug, so that the larvae partly concealed in the

surface of the soil may easily be found with the aid of a magnifier and removed with forceps. In a few cases it became necessary to transplant the prothallia to newly prepared culture dishes. After a thorough renovation of the greenhouse in midsummer this pest disappeared. Attacks of fungi, which occurred occasionally in a few cultures, gave little trouble. As soon as a moldlike growth appeared, the infected part was removed and destroyed. During the entire four years but one small culture was lost because of fungal growth.

Observations

MATTEUCCIA NODULOSA.—Although the behavior of the prothallia of the two species was similar in many respects, that of each will be given separately. As stated in the foregoing, as soon as the more vigorous specimens in the stock culture were large enough to handle easily (2–4 mm. diameter), they were carefully transplanted to separate cultures, being spaced 2 or 3 cm. apart. If the plants touched one another or became crowded because of increase in size, a number of individuals were again transplanted to other saucers. Text fig. 1 illustrates the method of culture, and shows the prothallia four months old when grown under these conditions. In cultivation the object desired was to grow the plants upon earth under the most favorable conditions that could be provided. Only the more vigorous and rapidly growing specimens were selected in every instance. Anyone who has examined fern spores carefully, and who has grown prothallia extensively, is impressed with the marked variation in the size of the spores; the readiness with which some germinate, and the rapidity and vigor of growth of certain prothallia. Presumably the larger and more rapidly germinating spores are those that produce the more vigorous prothallia.

At the end of about four months from the date of sowing (October 11, 1922 to February 28, 1923), the largest prothallia had attained a width of 15 mm. (text fig. 1). These plants had a relatively broad, massive midrib (archegonial cushion), and broad, rounded wings. At this age the wings are noticeably ruffled or wavy. In a number of plants there developed from the anterior margin of the wings, near the apical sinus, small thin, colorless, scalelike outgrowths, which generally curved upward and backward, or became variously twisted.

They were chiefly one cell in thickness and contained little or no chlorophyll. In the photograph they appear white and are easily recognized. After a time such scalelike outgrowths wither and disappear. They appeared now and then in older prothallia, but with no marked regularity. In character they suggest the minute pinkish scales borne along the margins of the thallus of *Marchantia*, although

FIG. 1.—*Matteuccia nodulosa:* culture of prothallia four months old, grown upon woods earth; natural size.

less uniform in size, and appearing much less regularly and in smaller numbers.

Text fig. 2 shows eight prothallia of the same culture when seven months old. In this culture, the only one in which the phenomenon occurred, a few plants developed a spinelike outgrowth from near the apical sinus (the central plant in the row of three at the right). These outgrowths were about 5 mm. in length, cylindrical or somewhat flattened, and tapering somewhat gradually to a sharp point. In structure they consisted of narrow, elongated parenchyma cells poor in cytoplasm. Near the center of each of those sectioned with the

microtome was a short strand of slender spiral tracheids three cells in width and four cells in length. The strand of tracheids was located at about one-third the distance above the base of the spine. No tracheids were present in the base of the spine, although that was its thickest part, nor in the midrib from which the spine developed. In a short time the spinelike outgrowths withered and disappeared, and no others were developed on the same plants afterward.

FIG. 2.—*Matteuccia nodulosa:* Prothallia seven months old; central plant in row at right shows spine-shaped outgrowth; natural size.

Of the plants grown, but three developed similar outgrowths. They were the only suggestions of anything that might be regarded as a tendency toward an apogamous structure. In the absence of a knowledge of their complete history and histological structure, one might easily be led to conclude that apogamy occurred here. That the structures withered in a comparatively short time, and occurred in but few instances, does not justify, from this evidence alone, the conclusion that apogamy occurs in *Matteuccia nodulosa*. These cases have convinced the writer that the presence of tracheary tissue alone in the prothallia is not proof of the existence of apogamy. If the prothallia of *M. nodulosa* had any tendency to develop apogamous

sporophytes, favorable conditions were certainly present for them to do so. The larger prothallia of text fig. 2 show the beginning of the characteristically ruffled margins of the wings, and the appearance of the heart-shaped proliferations that spring from the surface and margins of both young and old parts of the plants.

When the prothallia of *M. nodulosa* have attained the sizes of those of text figs. 1 and 2, from seven to eight months from the time of sowing the spores, their subsequent development results in one or the other of two forms of growth described by earlier observers. They may branch dichotomously, as in figs. 1, 2, and 6, or they may give rise to very irregular expansions, as seen in fig. 7. In some cases they produced dichotomous branches sparingly, with the result shown in fig. 4, although in this and in similar cases proliferations are beginning to develop at the proximal ends. Prothallia with the growth habit of fig. 1, are of theoretical interest because of their resemblance to certain dichotomously branched thallose liverworts. At a glance the plants might easily be taken for such liverworts as *Pellia*, a fact frequently mentioned by the earlier observers. At the end of four months' growth this plant had branched dichotomously, and subsequently each of the two shoots branched again in the same manner. A massive midrib, or archegonial cushion, leads to each growing end, which is relatively broad. The margins or wings of each shoot are quite regularly ruffled, and the branches tend to overlap to some extent. On the under side of the midrib or midribs, numerous archegonia are regularly and continuously developed. At the margins of such prothallia as shown in figs. 1–6, numerous proliferations are formed, which bear antheridia in great numbers. These proliferations are too small to show distinctly in the photographs. In addition to the marginal antheridia-bearing proliferations, small granular ones appear on the dorsal surface of tne midrib, especially of the older parts. At the older proximal ends of figs. 6 and 7 may be seen rounded, wartlike outgrowths of a granular appearance, covered with antheridia. In some cases, when the older parts of the midrib had begun to turn brown as if dead, small antheridial proliferations developed from the dark brown surface. While the older parts of the midrib appear dead at the surface, the cells below the epidermis remain meristematic for a longer time. Death of the older proximal

parts of the prothallia is slow and gradual when growing conditions are the best, and may not begin until the close of the second year. The behavior of the plant shown in fig. 6 differed somewhat from that of fig. 1. At the age of ten months it consisted of six definite growing points, resulting from dichotomous branching.

The second growth habit is to be seen in figs. 3, 5, and 7, where the branching is much less regular than dichotomy, the result being a very irregular form. Fig. 3 represents the plant at the age of ten months, while fig. 7 is the same plant at the age of one year. In so far as the writer could ascertain, these plants were not injured in any manner. A comparison of figs. 7 and 7*a* will indicate strikingly the size that may be attained in one year's growth under favorable cultural conditions when sporophytes are not developed. The plant represented in fig. 7 is the result of both dichotomy and larger lateral proliferations. Such lateral proliferations spring either from the margins of the wings or from the midrib. In the literature they have been referred to as adventitious shoots. In this, and in other similar prothallia, numerous small, heart-shaped proliferations are developed, in addition to the larger and more vigorous shoots. Most proliferations that attain any considerable size begin as heart-shaped outgrowths. Fig. 7*a* is a prothallium about six weeks old, bearing a young sporophyte consisting of the first visible leaf and root. Under ordinary conditions prothallia usually produce sporophytes when 2–4 mm. in diameter. The area of the prothallium in fig. 7*a* is close to 9 sq. mm., while that of fig. 7 is 1700–2000 sq. mm., not counting the smaller proliferations.

The plant shown in fig. 8 was photographed a little less than natural size when two years and three months old. Up to this time it had not grown as rapidly as some others, but from this time on growth was vigorous. The photograph was taken January 24, 1925. During the previous December the plant grew slowly because of unfavorable greenhouse conditions. In its earlier growth it developed three shoots which grew in as many different directions. The oldest parts have died, the three shoots being still connected by the dead strips of old midribs. In all prothallia the older parts die and decay sooner or later, as in liverworts. In some cases the death of the old-

est parts did not begin until about the end of the second year, but there is much variation in this feature, as would be expected.

In the plant in question (fig. 8) the lower left part consists of five vigorous shoots or branches, each with a relatively broad, massive midrib, and much ruffled margins. The part second in size (the upper in the figure) has three branches, and the smallest part (at the right) has two distinct growing points. In these shoots the branching seems to have been of the dichotomous type, although lateral shoots arise behind the growing edge or apical sinus. The growth habit here is almost a perfect imitation of that of a thallose liverwort. In this, as in almost all other prothallia of this species, many lateral proliferations arise from the margins of the ruffled wings. The smaller of these bear numerous antheridia. Archegonia are confined largely to the under surface of the midrib, while few or no antheridia may develop there. For this reason fecundation is very easily prevented.

Three months later (April 24) the three parts, continuation shoots, were completely isolated, the proximal portions of the midribs having completely decayed. The smaller part grew less rapidly than the other two. The surface now covered by the three shoots, with the exception of the central area resulting from the death of the central portion, measured 5.5 by 7.5 cm.; that is, from the spore a prothallial growth equal to those dimensions had developed in two and one-half years. This culture was followed carefully in every detail, in so far as that was possible without injury to the resultant growth. From April to November 1925 these continuation shoots grew rapidly in the manner described, so that by the end of the third year, November 11, 1925, the resultant growth formed a fairy ring or ellipse whose minor axis measured almost 7 cm. and the major axis about 9 cm. (text fig. 3). As will be seen, the shoots are extensively branched, the branches tending to overlap and crowd one another. The growth as a whole continued to spread and enlarge radially in a centrifugal direction, and also centripetally toward the central space left by the death of the older parts. On October 11, 1926, four years from the date of sowing the spores, the continued growth of this one prothallium had spread over an area somewhat circular in outline, 12 cm. in diameter (text fig. 4). The method of branching is

chiefly dichotomous, the older proximal portions gradually dying. I have spoken of the branching as the dichotomous type, for it is the growing point, or apical sinus, that bifurcates in a manner described by earlier observers. In addition, branches of the prothallia of the ferns in question arise frequently also by proliferations from the margins and from the upper and lower sides of the midrib.

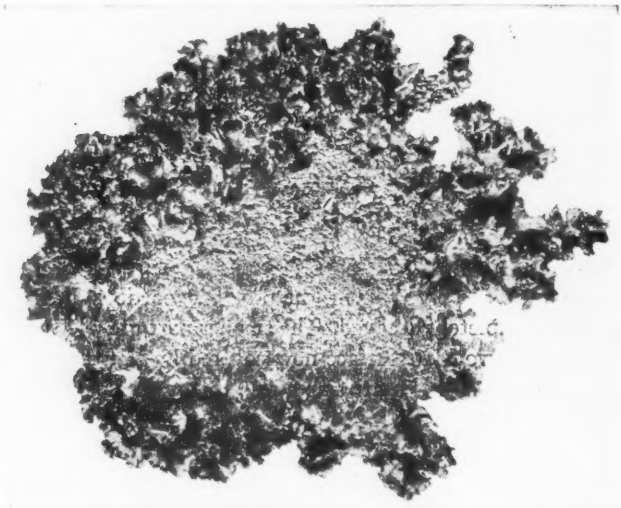

FIG. 3.—*Matteuccia nodulosa:* fairy ring of continuation shoots developed from single prothallium at end of third year; natural size.

As may be seen from the photographs with a certain degree of clearness, the more vigorous shoots are about 10 mm. wide, with midribs 3–4 mm. wide. When the shoots are not crowded they become broader, as shown in text fig. 4. The entire growth is now represented by the three large separate masses, all the shoots of each still histologically connected. In this culture the continuation growth consists of numerous shoots with definite midribs. In places the shoots crowd and overlap to a large extent. This leads to a more rapid death of older parts. Text fig. 5 shows the habit of growth which is typical of the continuation shoots of *M. nodulosa*, at the end of the fourth year. It represents the result of one of the shoots of a parent

prothallium which became independent through the death and decay of older proximal parts.

One prothallium of *M. nodulosa*, therefore, if kept growing for four years on soil and not allowed to develop sporophytes, will produce a plant body approximately equal to a surface 120 mm. square, 14,400 sq. mm., or 1600 times as large as a prothallium which bears a

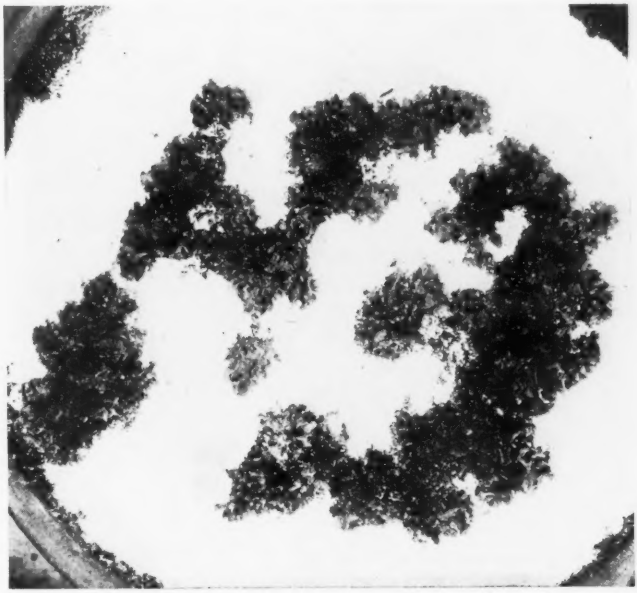

FIG. 4.—*Matteuccia nodulosa:* surviving continuation shoots resulting from single prothallium at end of fourth year; $\times\frac{2}{3}$.

sporophyte under ordinary conditions. The actual mass of the plant body produced is probably much greater, owing to the overlapping of the many lobed and ruffled shoots. Each shoot seems to be capable of continuing growth in somewhat liverwort fashion for an indefinite time.

OSMUNDA CLAYTONIANA.—The general behavior of the prothallia in this species was found to be similar to that in *Matteuccia nodulosa*. As pointed out by CAMPBELL (1), they are more elongated than

those of the Polypodiaceae, bearing a resemblance to the liverwort *Dendroceros*. The midrib projects strongly from the lower surface. The irregular branching and the presence of dichotomy, as stated by this author and by GOEBEL, have been referred to in preceding paragraphs.

Text fig. 6 illustrates the habit of growth and the size attained at the end of the first seven months. Even in prothallia of this age the resemblance to such liverworts as *Pellia* and *Dendroceros* is striking. As a rule they do not become as broad and as massive as the prothallia of *Matteuccia nodulosa*. The difference may be seen by comparing text figs. 1 and 6. In fig. 9 are shown four of the plants from

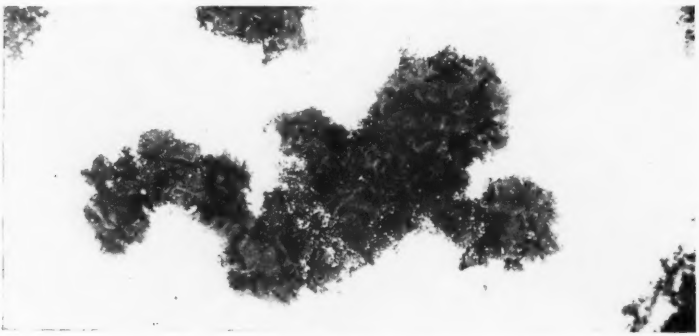

FIG. 5.—*Matteuccia nodulosa:* part of continuation shoots resulting from single prothallium at end of fourth year; natural size.

the culture represented in text fig. 6, at the age of fourteen months. GOEBEL (3) speaks of a four-year old prothallium of *Osmunda regalis* that had attained a length of 4 cm. The largest plant in fig. 9 measured nearly 3.5 cm. in length, and the very unusual prothallium shown in fig. 11 had a length of 5 cm. at the age of twenty months. Here, as in *M. nodulosa*, one recognizes two types of development: that of a broad, irregular expansion, which is comparatively rare, however, and the less branched habit in which the larger shoots or proliferations are fewer. The former type of development is shown in figs. 10 and 11, and the latter in fig. 9. Dichotomous branching occurs in old prothallia, but it is less frequent than in *M. nodulosa*. The two shoots resulting from the dichotomy grow unequally as a

rule, so that one becomes much larger than the other. As a result, therefore, we do not have the regular symmetrical habit seen in figs. 1 and 2, but that which is more like fig. 10.

The most remarkable plant in the culture of *O. claytoniana*, as regards habit and vigor of growth, is represented in fig. 11. This figure is a photograph, natural size, of the plant when twenty months old. It consisted of numerous large and small branches, more or less crowded and overlapping. All the midribs were alive and connected histologically, so that the whole consisted of one much branched

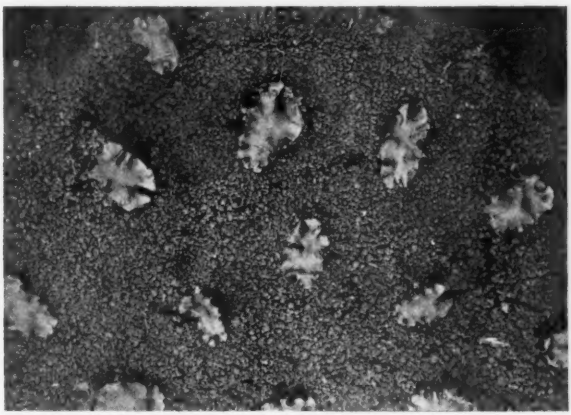

FIG. 6.—*Osmunda claytoniana:* several prothallia from culture seven months old; natural size.

prothallium. Each larger branch has developed many smaller, heart-shaped proliferations, which give the entire plant the appearance of a thousand or more ordinary prothallia massed together. The larger shoots bear numerous archegonia on the under side, and in some cases on the upper surface as well. Many of the smaller proliferations are thickly beset with antheridia. In older prothallia few or no antheridia are developed on the under side, near the archegonia. Because of this fact fecundation took place very rarely, and its prevention altogether was easily accomplished. When sporophytes appeared occasionally in later stages of this and other plants, they were usually found on the smaller and younger proliferations.

The nature of the larger shoots of this plant is shown by those that extend some distance beyond the margins of the mass. The extreme ruffling of the wings of the shoots is plainly seen in these extensions, as well as in the central part of the plant. The photograph (fig. 11) was taken January 24, 1925. Some weeks after that date the central part of the plant began to turn brown and die. Death at the center continued gradually, and after a few months the plant consisted of a somewhat circular expansion with a dead, brown center. Following the death of the older central part, the shoots continued to grow in different directions, spreading toward the periphery and in the direction of the center. At one time a moldlike fungal growth appeared on a part of the dead central mass, and the affected portion of this central tissue was carefully removed. The mold did not reappear at any subsequent time in this culture.

With continued growth and branching of the main shoots, along with the death of their oldest proximal parts, the entire surviving continuations of this plant at the end of three years and five months, as they appeared in the culture, are shown in text fig. 7. There are three irregular continuation masses or expansions, that have become completely isolated through death of the older parts by which they were once connected in one piece. The extremities of the largest of these irregular continuations will doubtless become disconnected by death of older parts at a later date. At the present writing the many shoots of these expansions show a fresh, vigorous growth. If the larger of these shoots were isolated carefully, they would be seen to resemble closely in habit the younger prothallia shown in fig. 9. The under side of each midrib continues uninterruptedly to develop numerous archegonia. Antheridia, although produced sparingly or not at all on the under side, are present in large numbers at the edges and on the surface of the small lateral proliferations of the wings. These antheridia-bearing proliferations, often very irregular in form, are frequently as small as, or smaller than the grains of white sand used in covering the bare soil of the culture before the photograph was taken. To the unaided eye such proliferations are often of a paler or yellowish green color, and when numerous and closely aggregated they appear somewhat as granular masses.

The continuation shoots of the four-year old cultures derived

from a single prothallium show about the same habit as that in fig. 9. Many are a little larger than those in this figure, with a midrib 2–3 mm. in width, and a total breadth of 7–8 mm. They contrast strikingly with the largest continuation shoots of *M. nodulosa*, in which the total width may exceed 12 mm., the midrib measuring 3–5 mm. An especially noticeable and characteristic feature of the continua-

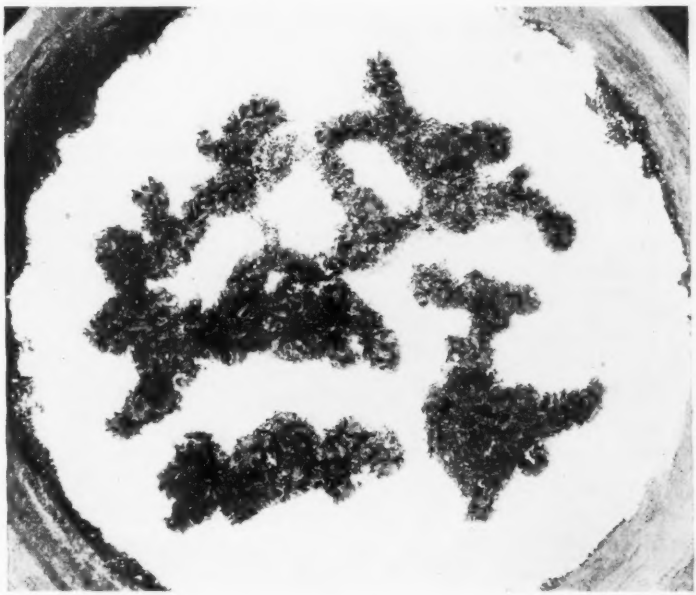

FIG. 7.—*Osmunda claytoniana:* continuation growth developed from prothallium at end of three years and five months; ×¾.

tion shoots of *O. claytoniana* is the pronounced ruffling of the wings, which in some cases may stand up at right angles to the midrib. The edges of some of the lobes, therefore, point vertically upward. In such cases the lobes on opposite sides conceal the midrib. Under such circumstances the upper surface of the midrib will frequently develop a row of minute wartlike protuberances which bear archegonia. These are developed also on the under side of such midribs; antheridia, however, appear on the uneven edges of the ruffled

wings. The edge of each lobe of the ruffle is uneven, and it is mainly from the margin of the uneven edges that antheridia develop. Archegonia frequently appear on the upper side of the midrib in certain prothallia when such part is not shaded by the upstanding or overarching wings. In a few cases, in which crowding caused certain shoots to grow upright, with the growing point extending straight up into the air, both archegonia and numerous brown rhizoids appeared on both surfaces of the midrib.

In the two species under discussion there seems to be no reason to believe that the continuation shoots resulting from one prothallium at the end of the fourth year could not continue to grow for an indefinite time if kept free from insect pests, from the more vigorous competitors that gain a foothold in the cultures, and from parasitic fungi. Although the plants of some of the cultures have died, as was to be expected, many have been successful, and the continuation shoots of these give at the present no indication of impaired vigor.

Endophytic fungus

An endophytic fungus has been found to be present in the following species of the ferns with green prothallia, as reported by CAMPBELL (**2**). *Marattia Douglasii* Baker, *Kaulfussia aesculifolia* Bl., *Angiopteris evecta* Hoffm., *Gleichenia polypodioides* Sm., *G. dichotoma* Willd., *G. laevigata* Hooker, *G. pectinata* Presl., and *Osmunda cinnamomea* L. To this list the writer is now able to add *Osmunda claytoniana* L.

CAMPBELL states that in *O. cinnamomea* the endophyte appears commonly, but it could not always be found. In three-year old prothallia of *O. claytoniana* the writer found an endophytic fungus in the older tissues. Of the three specimens imbedded and sectioned, the fungus was present in two but absent from the third. Until the cultures were four years old I did not wish to sacrifice the plants for histological study; however, a somewhat more careful study of their structure will be made in the future. In the four-year old prothallia of *Matteuccia nodulosa* no endophyte has been found in any of the plants examined. It seems probable that the endophyte may be present in this species also. In prothallia of *O. claytoniana* the fungus seems to be confined to circumscribed areas in the midrib. It does not occupy a uniform layer nearer the ventral surface, but its distri-

bution in the specimens observed suggests that of the colonies of *Nostoc* in the gametophyte of *Anthoceros*. The region containing the fungus may be midway between the upper and lower surfaces of the prothallium, or nearer the under surface.

The hyphae are relatively large, tubular, branched, and multinuclear. The structure and appearance of the fungus agree closely with that figured for *O. cinnamomea* by CAMPBELL (2). Conidia were not found. In cells containing the fungus the plastids are strikingly different from those in the neighboring cells free from the endophyte. In the former the plastids tend to aggregate into irregular, lumpy masses. They do not contain any starch inclusions, but possess dense and very finely granular contents, which stain deeply and uniformly throughout. With the triple stain of anilin safranin, gentian violet, and orange G (dissolved in clove oil), these plastids retain the safranin strongly, but the violet is easily washed out. As a rule the infected cells are very poor in cytoplasm. The nuclei in some cases seem normal, but in many cells near the center of the infected region the nuclei are ameboid in shape, and appear abnormal in structure. The contents of the fungal filaments differentiate readily in staining, the small nuclei being well defined, and the granular cytoplasm retaining the blue color a little more strongly than the red.

The cell walls of the older parts of the midrib of the prothallium are much thicker than those near the growing point, and are perforated by numerous pits varying much in size. The pits are round, oval, or elliptical; some are elongated and slitlike. They are not arranged in any definite order in the wall. The fungal filaments pass from cell to cell through the larger pits. The fungus was found in some of the rhizoids, so that it seems probable that it gains entrance partly through the rhizoids. Whether we have here a case of parasitism or one of symbiosis is a question. The prothallium does not seem to be injured in the least by the presence of the fungus, and it is difficult to believe that any benefit is received from the endophyte. That the fungus receives food from the prothallium can scarcely be doubted.

Discussion

In comparison with the gametophyte of the Hepaticeae, the prothallia of the Polypodiaceae and Osmundaceae seem to stand out conspicuously in two respects, namely, the longer duration of life,

and the capacity to produce plural sporophytes on the part of the gametophytes of the Hepaticeae. In the Hepaticeae as a rule the gametophyte is polycarpic, or capable of producing plural sporophytes; while in the Polypodiaceae especially, polyembryony is rare under conditions out of doors. Under experimental conditions, however, it has been demonstrated that the gametophyte may readily produce a number of sporophytes (MOTTIER 4).

Under ordinary conditions the gametophyte produces one sporophyte and dies; it is exhausted by the rapidly growing sporophyte. Prothallia of *Matteuccia nodulosa* may produce a sporophyte at the end of a month or six weeks, and when the plants are no larger than 2 mm. in width. This rapid "turn over" in the Polypodiaceae enables the self-nourishing prothallia to escape competition with more vigorous growths out of doors. Under cultural conditions growing prothallia for short periods of time is an easy task, but if prolonged throughout a period of years, the holding in check of more vigorous competitors requires incessant care. Prolonged life is possible only when exhaustion by sporophyte production is prevented, and when stronger competitors, such as mosses, etc., are kept in check.

As mentioned in a preceding paragraph, GOEBEL found that a prothallium of *Osmunda regalis* four years old had attained a length of 4 cm. In my cultures the prothallia of *O. claytoniana* easily attained a length of 3.5 cm. in fourteen months. This size is reached by the more vigorous plants if not crowded and if grown on soil. GOEBEL gave no data in regard to the method of cultivation. He seemed to be of the opinion that prothallia of the Filices would continue growth indefinitely if the production of sex organs could be prevented. In my cultures the production of antheridia and archegonia did not seem to impair the vigor of growth in any perceptible way. Throughout the entire time the prothallia, and their continuation shoots, produced sex organs continually. That which is necessary is the prevention of the production of sporophytes, in order that the prothallia may continue indefinitely. Referring further to the duration of life, GOEBEL (5) states:

> Prothalli, upon which the act of fertilization has not been performed, may grow for a long time, but in them sooner or later phenomena of senescence appear, showing either in abnormal conformation or in the development of adventitious shoots.

In the light of the behavior of these cultures, I should not be inclined to look upon the formation of lateral proliferations or dichotomous branching as an indication of senescence, since such proliferations or shoots arise not only from the older proximal parts of the thallus, but from the growing ends as well. The continuation shoots resulting from one prothallium at the end of the fourth year, if not injured or too much crowded, continue to grow normally, showing the precise habit of one-year old plants. In *M. nodulosa*, for example, certain vigorous shoots of the four-year old cultures branch dichotomously and seem to be normal in every way. Some resemble closely the younger prothallia shown in figs. 1 and 2. Whether the habit of dichotomous branching is manifested in certain prothallia only, as an inherited character, or merely as an individual variation due to some external cause, cannot be stated with any degree of certainty.

The cells at the growing point of the four-year old shoots do not seem to differ in appearance structurally from those of younger plants; however, old age and decay result regularly in the older proximal tissues. At the end of the second year, and sometimes earlier, the proximal parts of the plants begin to turn brown, shrivel, dry out, and decay. From such older parts it very frequently happens that fresh proliferations develop, sometimes as larger shoots, sometimes as small irregular outgrowths replete with antheridia. Such outgrowths develop also from the younger parts of the thallus. It was observed frequently that fresh green proliferations sprang from the upper surface and edges of older proximal parts of midribs that were dark brown in color and apparently dead. Such older parts retain the power of developing local meristematic regions in a high degree. Sooner or later, however, the older parts gradually die, and this death progresses slowly in the direction of the growing point. Because of this fact the several branches become in time completely separated histologically. The rapidity of such death and decay is determined very largely by environic conditions. GOEBEL did not state whether his four-year old prothallium of *Osmunda regalis* 4 cm. in length had undergone any death or decay at the proximal end. Some of my plants attained a length of 3.5–4 cm. before the end of the second year, but after this time the proximal ends almost invariably began to die and shrivel up. Inasmuch as fresh shoots may

grow directly from the older tissues that have become brown on the surface, the interesting question arises as to what constitutes old age or senescence in such tissues.

As mentioned in a preceding paragraph, GOEBEL speaks of "abnormal conformation," or the development of adventitious shoots, as an indication of senescence in fern prothallia. In many cases abnormality or a marked departure of the ordinary form of the gametophytes resulted from what was looked upon as some external injury, or as the result of overcrowding. However, in both species studied certain departures in form and regularity of branching occurred from time to time in what seemed to be perfectly fresh and prolific plants, so that variations in form and the formation of shoots do not seem to indicate senescence.

One of the questions the writer hoped to answer through this study was as to how large a fern prothallium will become if not exhausted by the production of sporophytes or destroyed by some parasite or other external agency, if allowed to grow for a number of years. If no parts had died or decayed, a single prothallium of *Matteuccia nodulosa* and *Osmunda claytoniana*, in four years, under the cultural conditions described, and according to the development manifested in the cultures, would have covered a surface of 14,400 sq. mm. The largest continuous mass attained by one plant, without death of any part, is represented in fig. 11. Death and decay of old proximal portions set in near the close of the second year, and the surviving growth at the end of three and one-half years is shown in text fig. 7. The surviving growth of one plant of *Matteuccia nodulosa* is shown in text fig. 4. Many of the surviving shoots of these and other similar cultures are still growing as vigorously as younger prothallia, and unless they are destroyed by external agencies, there is no reason to doubt that they may continue indefinitely.

Summary

1. Prothallia of *Osmunda claytoniana* and *Matteuccia nodulosa* have been grown on soil for over three and one-half and four years respectively, under cultural conditions that prevented the production of sporophytes. When an occasional sporophyte appeared, it was amputated in such a manner as not to injure the gametophyte.

2. Prothallia thus grown branched both dichotomously and by the production of lateral shoots or proliferations. Proliferations, large and small, develop from the margins, from both surfaces of the midrib, and from the older proximal tissue, as well as from the growing point or apical sinus.

3. In older prothallia archegonia may be developed from both upper and lower surfaces. In such prothallia antheridia are borne chiefly on small marginal proliferations, or on small granular protuberances on older parts.

4. In some cases small protuberances are produced along the midrib upon the upper side. Such protuberances bear many archegonia.

5. The cell walls in older parts of the midrib are relatively thick, and are marked by numerous pits varying in size.

6. An endophytic fungus is present in certain circumscribed areas of older midribs of *Osmunda claytoniana*. The fungus has a tubular, branched, multinucleated mycelium. The hyphae may pass from cell to cell through the large pits in the cell walls. No fungal spores were observed.

7. A spinelike process developed from near the growing point in a few prothallia of *Matteuccia nodulosa*. These dried up as the prothallia grew older.

8. Apogamous sporophytes did not develop in any case.

9. If sporophytes are not produced, the continuation shoots of the prothallia seem to be able to continue growth indefinitely.

It gives me pleasure to acknowledge my indebtedness to my colleague, Dr. PAUL WEATHERWAX, for making the photographs used in the illustrations.

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EXPLANATION OF PLATE X

All figures were prepared from photographs. Figs. 1–8, *Matteuccia nodulosa;* figs. 9–11, *Osmunda claytoniana.*

FIGS. 1, 2.—Prothallia eight months old, showing dichotomous branching and typical ruffled character of wings and broad midrib; natural size.

FIG. 3.—Prothallium ten months old, showing more irregular mode of branching; $\times 1\frac{2}{3}$.

FIG. 4.—Prothallium ten months old which had not branched dichotomously; several proliferations have developed from older tissue of proximal end; $\times 1\frac{2}{3}$.

FIG. 5.—Prothallium which has branched irregularly; $\times 1\frac{2}{3}$.

FIG. 6.—Prothallium ten months old having six growing points resulting from dichotomous branching; at proximal end small granular-like protuberances bearing antheridia have developed; $\times 1\frac{2}{3}$.

FIG. 7.—Same plant as fig. 3, when one year old, showing irregular branching; wartlike protuberance at proximal (lower) end bears numerous antheridia; $\times 1\frac{2}{3}$.

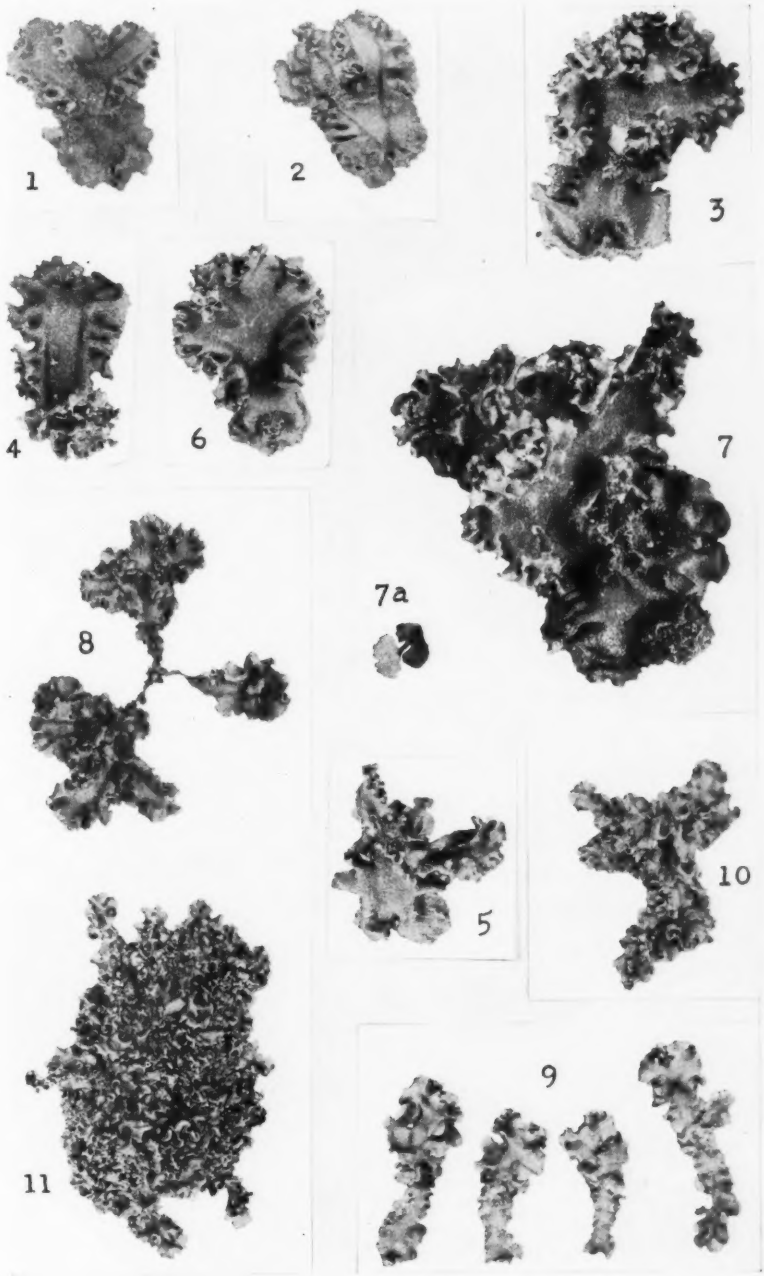
FIG. 7*a*.—Prothallium six weeks old, with sporophyte attached; $\times 1\frac{2}{3}$.

FIG. 8.—Prothallium two years and three months old, consisting of three large branches still connected by decaying midribs; branching regularly dichotomous; natural size.

FIG. 9.—Four prothallia of *Osmunda claytoniana* from same culture, fourteen months old, showing growth typical in this species; natural size.

FIG. 10.—Prothallium twenty months old, somewhat irregularly branched; natural size.

FIG. 11.—Unusual prothallium of *O. claytoniana*, twenty months old; natural size.



MOTTIER on FERN PROTHALLIA

species which normally occur in a certain habitat may be entirely absent throughout a season. This is true to a much less degree for the pteridophytes and spermatophytes. It is necessary, therefore, to accumulate, compare, and correlate evidence obtained through several years of observation in order to obtain a picture of a truly representative community of fungi. Eight years of observation have accumulated far too little evidence for conclusions concerning many of the successions discussed herein. The differentiating points for the identification of fungi are fewer, less definite, and more variable than for the spermatophytes. *Pleurotus ulmarius* illustrates their variability. Several standard descriptions have been made of this plant, none of which admits the scaly stemmed form so prevalent on the trees of our city streets. Forest forms have the smooth stem which corresponds to the standard descriptions. This is but one of the numerous examples in the taxonomy of fungi. The taxonomy must be mastered before the ecological study can be attempted. Figs. 1 and 2 present keys devised for the purpose of facilitating the taxonomic study. The peculiar nature of the differentiating characters of the carpophores makes such a key invaluable, especially to the beginner. Comparative sizes used in this discussion can be checked with fig. 2.

Seasonal occurrence

The flowering plants have a distinct seasonal sequence. Spring is represented by characteristic vernal species. The summer plants of the herbaceous type are of different aspect, and the autumn plants are larger than either the summer or the spring plants. The change of flora of the higher plants is paralleled by fungi, which present a different flora each season. *Morchella* and *Peziza* are representative of the spring, *Russula* and *Lactarius* of the summer months, and the autumn flora is represented by a tremendous number of species. Many which occur in the spring may be found throughout the summer, and some continue into the autumn; namely, *Collybia velutipes*, *C. dryophila*, *Pluteus cervinus*, and others. Scarcely any member of the genus *Morchella* is found at any other time than spring, and the same is true of *Peziza* with but a few exceptions. A greater number of forms represent the summer months, which are dominated by numerous species of *Russula* and *Lactarius;* these continue into

September, but are seldom found after October 1. Other summer genera represented to a considerable degree are *Amanita*, *Hygrophorus*, *Mycena*, *Omphalia*, *Psalliota*, and *Hypholoma*. *Tricholoma*, *Hebe-*

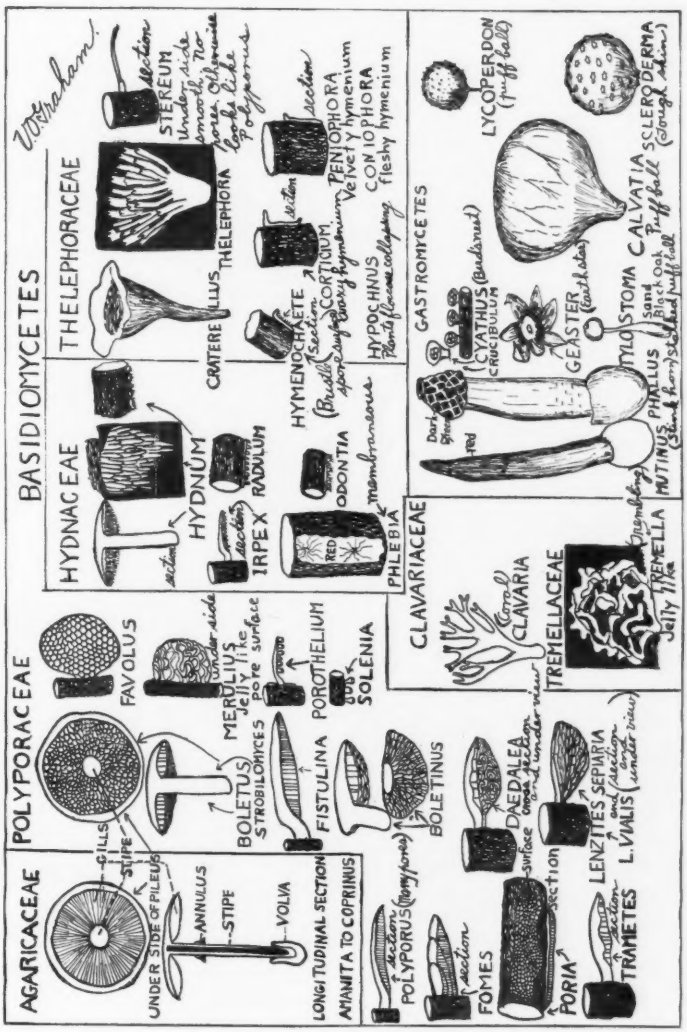


FIG. 1.—Diagram describing differentiating points of the divisions of the Basidiomycetes

loma, Cortinarius, and other deep forest fungi are the dominating inhabitants of the autumn, while *Polyporus, Fomes, Trametes, Daedalea,* and *Lenzites* may be found in the winter. The winter spe-

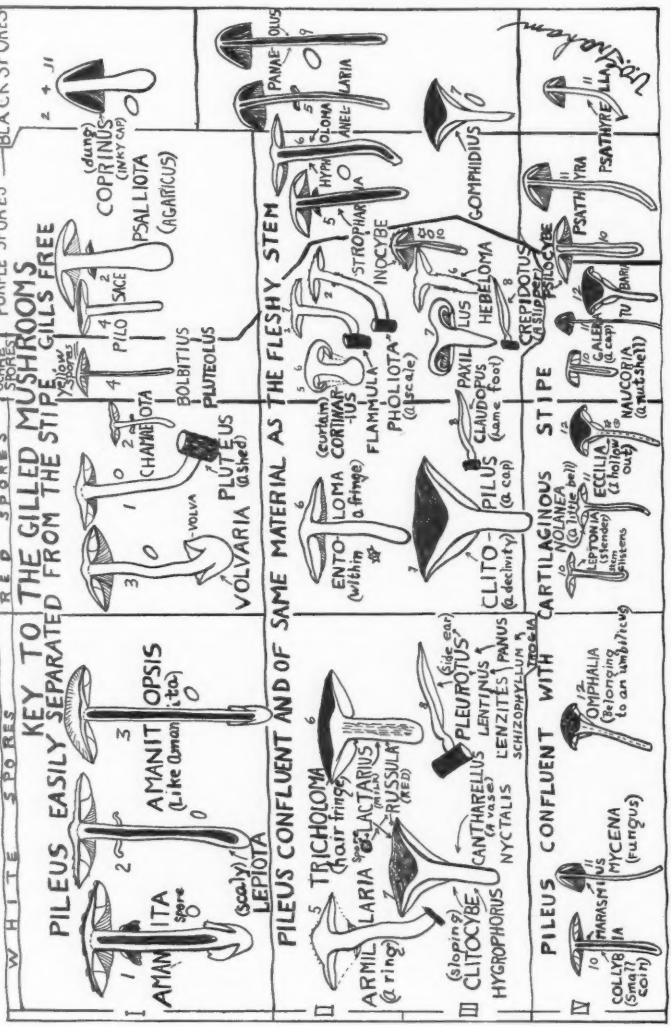


FIG. 2.—Key to the Agaricaceae, reading both vertically and horizontally

cies produce their carpophores in the warmer seasons and persist through the winter. *Collybia velutipes*, a soft species, produces carpophores at any season, but this is an exceptional case.

The abundance of fungi is also affected by the season. The spring and autumn species differ as to the type of habitat in which they are most abundant. In any habitat the humus supply is fairly constant, while soil temperature and moisture content are variable. Fungi are abundant in the shady forest in autumn but almost absent in spring, while in the pasture and open forest they are present in spring but absent in autumn. The pasture and sunny hillside, in an open forest, are abundantly supplied with moisture by the spring rains, but such a habitat is very dry in the summer and autumn. The shady forest floor with its leafy carpet retains moisture, but the retardation of the rise of temperature causes a paucity of fungi in spring and early summer. The ascent of soil temperature is greatly retarded in spring, while temperature decline is retarded in autumn; consequently fungi do not occur in a shady forest habitat until late in summer, but continue to occur until long after severe frosts in autumn. On the other hand, the soil of the pasture and other open habitats has an early rise of temperature, with moisture supplied by the spring rains, while the decomposing grass and dung furnish the necessary organic material. Such a habitat furnishes the majority of spring fungi. Due to the absence of moisture after the period of spring rains, the open grassy habitat is barren of fungi, but if rains are abundant throughout the summer, as sometimes happens, *Psalliota campestris*, *Galera tenera*, and species of *Panaeolus* will occur. The summer is more often very dry, hence xerophytic conditions prevail and fungi are absent. The presence of the three factors, sufficient humus, abundant moisture, and favorable temperature are necessary for the development of numerous carpophores.

The production of fungi is more retarded than the seasonal rise of temperature. The longest day of the year is June 21. Were it not for modifying factors this would be the hottest day, but retarding factors cause the season to be delayed and we conceive of the longest day of the year as the beginning rather than the middle of the summer. Because of the time required for growth, plants generally are retarded even more than the rise of the air temperature. Some flow-

ering plants because of their ability to store energy in their subterranean parts are able to grow quickly and bloom in the early spring. The growth process for fungi is much slower. We are accustomed to say "mushroom growth" when we refer to very quick growth, but the greater part, the mycelial development, is overlooked. Under most favorable conditions a rapidly growing species of *Coprinus* will develop carpophores in twelve days. Larger fungi may require months. The time consumed for the rise of the soil temperature, plus the time necessary for the growth of the mycelium, causes a great retardation of spring production of fungi.

Mycelial growth of less quantity is required to produce the smaller carpophores, but the time required for the production of the large carpophores is so great that they seldom occur in the spring. The spring species on the average are much smaller than the autumn ones, while those of the summer are of intermediate size. Fig. 2 shows the genera of fungi with cartilaginous stems to be much smaller than those of the other divisions. Such genera as *Marasmius*, *Mycena*, *Omphalia*, *Galera*, and *Collybia* are well represented in the spring, while *Russula*, *Lactarius*, *Amanita*, *Amanitopsis*, *Hygrophorus*, *Entoloma*, *Psalliota*, *Lepiota*, and *Panaeolus* are well represented in the summer. The larger species of these genera continue quite generally into September. This is a statement of a general observational conclusion and has many exceptions. *Tricholoma* is the most notable autumn genus in this region, but farther north *Cortinarius* becomes important. *Armillaria mellea*, so often attached to oak roots, is found in September and October in abundance; in November, after this species ceases to occur, *Tricholoma personatum* and *Hygrophorus Russula* push up their leafy covering as tufts on the forest floor. *Peziza coccinea* may be found in very late autumn and very early spring. Dr. W. B. McDOUGALL says that it is likely that it requires a rather low soil temperature, perhaps a very cold soil with a somewhat warmer surface, a condition that usually exists in March and occasionally in December.

Trips in June to Palatine, where there is an open forest on rolling ground, revealed an interesting spring community. On the xerophytic moss *Ceratodon purpureum* occurs a community of *Omphalia fibu-*

loides. Sometimes a dozen carpophores are present on a patch of moss 3 ft. in diameter. The habitat of this moss is very dry in summer, but the abundant spring rains furnish moisture in the early season. Such is a typical spring community.

At Morton Grove in June appears another spring community in the grassy open forest. *Marasmius oreades*, and sometimes *Morchella esculenta*, are present in numbers where the open habitat makes possible the early rise of the soil temperature, and the spring rains have supplied the moisture. Either an old orchard or a south hillside exposure in an open forest is a favorable habitat for a community of *Morchella*. In autumn the north exposure is the better fungus habitat. The south exposure is usually grassy, the north is often moss covered; these, however, are not the determiners. The deficient spring factor is likely to be heat, and the south exposure receives the rays from the sun more vertically. The deficient factor in autumn is likely to be moisture. This is best retained where evaporation is least, and evaporation is least where the sun's rays are indirect or none. The south face is warmer but drier than the north face. The warm south face with its abundant moisture from the spring rains is a favorable habitat for vernal fungi. The north face with the accumulated heat retained in the soil by the vegetable covering is a favorable autumn habitat, but it is too cool in the spring for fungus growth.

Unshaded sand swales in the dune area are favorable for a community of fungi in the spring, with *Inocybe caesariata* one of the dominating species of this habitat. Due to the ever present supply of moisture, the sand swale is a favorable habitat throughout the summer and autumn.

In summary, the spring communities have the following characteristics: (1) the fungus community grows in the open; (2) carpophores are small; (3) the fungi grow on moss, in grass, or on dung; (4) the growth is in a habitat which is very dry during the summer and autumn.

The alternation of a dry and wet period makes possible the study of the ability of certain habitats to retain moisture and continue to produce fungi when other habitats cease production. During the dry

August of 1925 the ravine slopes continued to produce fungi for more than a week after other parts of the forest had ceased their productivity. The dry August followed a moist period in July. The accumulated moisture is to be found in the deeper soil layers during the dry period following the wet. The moisture moves upward by capillarity, and downward and horizontally by gravity. The horizontal movement causes the ravine slope to remain moist long after the flat forest floor has become quite dry; consequently the ravine continued to produce an abundant supply of fungi. In September, following the dry period, heavy rains fell. The forests were visited with the expectation of an abundant supply of fungi, but few were found. Not until the rains continued for three weeks did the growth of the carpophores become normal for the time of year. Close examination of the data revealed that the successive trips brought a harvest of fungi the size of which was successive. The smaller fungi were observed on the early trips, the larger on the later trips. It seems evident that the dry period was of sufficient severity to stop the growth of the mycelium. The return of the wet period found this growth entirely dormant. The energy in the mycelium had been spent in the continued production of carpophores after the beginning of the dry period. Time was consumed in the accumulation of energy for the renewal of carpophore production. The small species which appeared first belonged to the genera *Mycena*, *Marasmius*, and *Coprinus*. Later came the normal production of larger forms of *Russula*, *Lactarius*, and *Tricholoma*.

Due to low temperature, such habitats as dunes and swamps do not produce fungi in the spring. Cold air being heavier than warm, the soil of the swamp is colder than that of the hilltop. Furthermore, the water of the swamp warms more slowly than the land. These are retarding factors in the spring temperature rise of the swamp. Fungi are abundant here in the late summer and autumn, when sufficient time has elapsed for the rise of temperature. The snow falls on the sand in the dunes, and the moving sand carried forward by the wind covers it. More snow falls and in turn is sand-covered. The snow prevents the rise of the temperature of the sand, and the sand protects the snow from the sun. Moving sand habitats thus have a retarded rise of temperature.

Soil

A comparison of the soil of a floodplain with a bog, in which the factors are either similar or in contrast, proves interesting. Water is present in abundance in both cases; temperature seems to be equally favorable in both habitats; the differences will be found to be chiefly in the soil. The peat bog soil is made up of layer upon layer of sphagnum, so that vegetable material is abundant. An examination of this may be made at Cedar Lake, Lake Villa, Illinois. The material above the water is about 6 inches thick. The floodplain at River Forest is a soil rich in alluvium, a soil which would be given high rank for corn growing, especially if the possibility of flooding were removed. The humus of the floodplain is not abundant. The alluvial particles which make up this soil are deposited during high water, and the accumulation of humus continues for but a short time when deposition is repeated. The peat bog produces fungi abundantly in late summer and autumn, while the young floodplain is about as unproductive in fungi as are the bare sands of the dunes. Floodplains sufficiently elevated to be free from flooding, or old floodplains where humus has accumulated for many years, produce fungi as any other similar flat habitat. If the area is a forest it proceeds to the climax, and as such will have such fungi as are found in climax forests.

The soil of the forest floor has abundant humus, composed of decaying leaves and wood. The amount of humus present is comparable with the amount in the sphagnum bog. Both soils produce an abundance of fungi, but the fungus community on the one is very different from the community on the other soil. This indicates that the chemical nature of the humus is an important determiner. The effect of the different chemical nature of humus is also illustrated by the soil made up of accumulated white pine needles near Furnessville, Indiana, where the fungus flora is entirely different from that of the bog or the ordinary forest. In any of the habitats where the supply of humus is great the number of fungi is notable.

Aeration is important. When the supply of humus is constantly below water none of the larger basidiomycetes are present. It seems that such a place is not favorable for the development of the mycelium, but when the humus is raised above the surface of the water, as

it is at Cedar Lake, air may be present and the growth of mycelium is considerable. Here water is abundant but it does not interfere with aeration.

Successional associations

The bog succession may be studied at Cedar Lake and at Mineral Springs. It is convenient in the study of the communities of fungi to consider them in relation to the successional associations of the higher plants. The succession of the higher plants may roughly be separated into the following stages:

1. Decodon verticillatus
2. Sphagnum, Drosera, Sarracenia, etc.
3. Bog shrub association
4. Larix laricina association
5. Betula lutea—Acer rubrum association
6. Climax association

The first four of these associations are represented in the Cedar Lake study. This lake is a depression in the Valparaiso moraine deposit. Northwest of Chicago numerous depressions in the moraine constitute what may be spoken of in a general way as lakes of the Fox Lake region. Many tamarack bogs occur. Near the western shore of Cedar Lake occurs one of those rare physiographic situations known as a growing bog. The *Larix laricina* association here is composed of small trees, and no evidence can be found to indicate either the presence or the approach of the next association in the successional order, the *Acer rubrum—Betula lutea* association. *Typha latifolia*, *Carex hystericina*, and *C. comosa* are near the shore. Two hundred feet into the lake is a line of *Decodon verticillatus*, the outer fringe of the developing bog. On this first association may be found a small reddish *Peziza* with a ciliated margin, but no other fungi are present. The second association composed largely of sphagnum is quite extensive. The mat of sphagnum is very thick, readily supporting the weight of one's body. The mat is several inches thick and the water several feet deep. The surface quakes when walked upon. Other members of this association are *Drosera rotundifolia*, *Sarracenia purpurea*, and several mosses. The predominating members of the fungus community are *Galera hypnorum* and *Omphalia fibula*.

Another association has developed on the sphagnum, a shrub association composed of *Vaccinium macrocarpon*, *Betula pumila*, *Salix candida*, and *S. pedicellaris*. In this association is found *Hygro-*

phorus miniatus var. *sphagnophilus* as the dominating fungus species. It is also found in bogs throughout the region, wherever the association of sphagnum is at all extensive. Near Merrillville, Indiana, a bog has developed into the fourth stage, which is dominated by *Pinus Strobus* instead of *Larix laricina*. Here in August *H. miniatus* var. *sphagnophilus* is present. In the Cedar Lake bog it is present from August to November, gradually increasing in numbers until the first of October. The most striking fungus in this association is *Boletus spectabilis*. *Entoloma nidorosum* and *Hebeloma crustuliniforme* form *sphagnophilum* were also present.

The fourth association contains the tree *Larix laricina*. The shrubs and sphagnum form the undergrowth, and in fact the soil for the trees. It seems improbable that *Betula lutea* and *Acer rubrum* will ever enter here. Considerable time must elapse before the filling in will be sufficient for this to be a normal habitat for such trees. Civilization is encroaching upon the place, and may interfere with the natural course of events before the development of the habitat makes possible the entrance of the association of *Betula lutea* and *Acer rubrum*. It is quite difficult to be sure that any of the fungi here found may be separated from the sphagnum and the shrub association and placed exclusively in the *Larix* association. *Russula fallax* and a species of *Lactarius* were found under the *Larix*, which did not occur in the undergrowth where the *Larix* was not present. The following data were collected September 30, 1926:

Association of higher plants	Community of fungi	No. of fungi
1. Decodon	Peziza	1
2. Sphagnum	Omphalia fibula	20
	Galera hypnorum	104
3. Shrubs on sphagnum	Entoloma nidorosum	20
	Hygrophorus miniatus var.	200
	Boletus spectabilis	10
	Hebeloma crustuliniforme form sphagnophilum	8
4. Larix laricina	Russula fallax	3
	Lactarius sp.	5

Fig. 3 shows the fungi found in the bog associations. These have their vegetative growth in the carpet of mosses, the prevailing one being sphagnum, but other mosses occur in sufficient abundance to

form a carpet somewhat independent of the sphagnum. On these moss carpets *Hygrophorus miniatus* occurs in great numbers.

The much older bog at Mineral Springs has the communities of fungi found in the older *Larix laricina* and *Betula lutea—Acer rubrum*

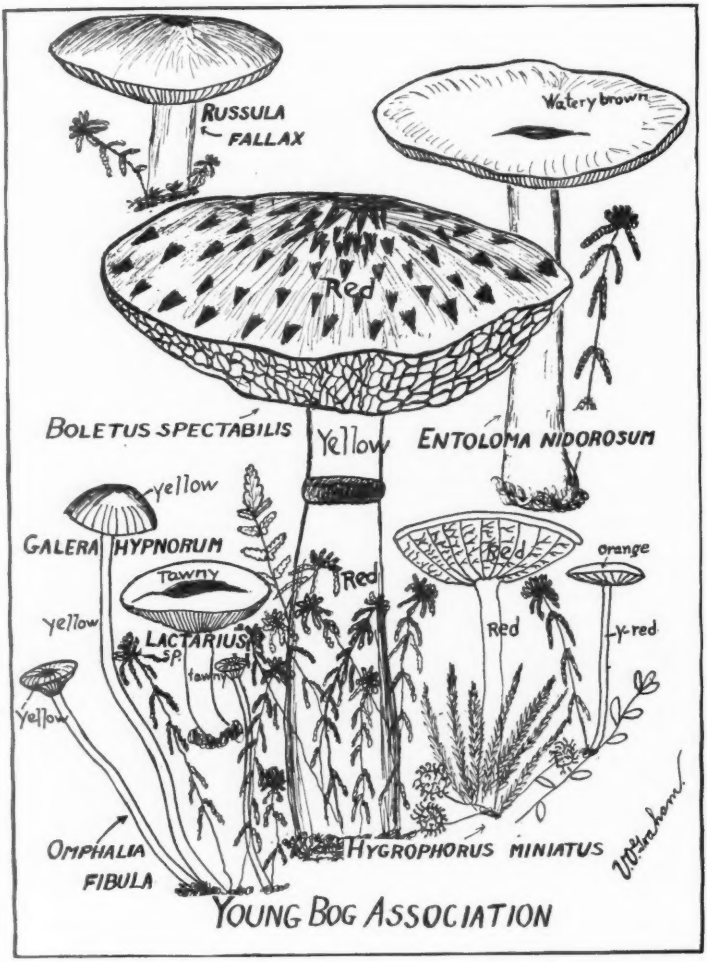


FIG. 3.—Young bog association, showing species of fungi most commonly found about October 1 on the floating land of Cedar Lake Bog at Lake Villa, Illinois.

associations. This bog is separated from Lake Michigan by dunes, its level being but little above that of the lake; consequently stagnant water is constantly present. Much care must be exercised here, as the habitats are adjacent to the xerarch succession of the dunes, and some overlapping of the fungus communities has occurred. The following is a somewhat uncertain list of the fungi found in the *Betula lutea—Acer rubrum* association: where *Larix* is present *Lactarius helvus*, *Cantharellus aurantiacus*, *Calvatia saccata*, *Hygrophorus speciosus*, and *Russula fallax* are found. These are also found in the association when *Larix* is absent, but in addition to them are *Collybia aquosa*, *Mycena sanguinolenta*, *Gomphidius maculatus*, *Russula delica*, *Hypholoma lachrymabundum*, *Lactarius camphoratus*, *Hygrophorus chlorophanus*, and *Mycena epipterygia*. Much of the soil material of the association of *Larix* is certainly present in the soil of the succeeding associations; the continuance of species from former associations may therefore be expected. The presence in the beech forest at Tremont of *Hygrophorus chlorophanus* and *Cantharellus aurantiacus* indicates that the soil substance contains some of the same materials found in the *Betula lutea—Acer rubrum* association at Mineral Springs. This may point to a method of estimating the past successional history of this forest.

The beech forest at Tremont adjacent to the dunes has a mixture of the fungi that are to be expected in the xerarch situations mixed with the bog-hydrarch successional climax. These cannot readily be separated except as they have also occurred in a previous association in one or the other of the successions. There is no other habitat in this area with the climax association developed from the bog.

The river-hydrarch zonation of associations is well shown at River Forest. The succession of associations in this series appears roughly in the following order: water plants, amphibious plants, *Salix nigra*, *Acer saccharinum*, *Fraxinus americana*, *Ulmus americana*, *Quercus rubra*, and *Acer saccharum*. This is only a rough division for the purpose of comparison. The most conspicuous feature concerning fungi in this series is their absence. The question arises why fungi are not as abundant in the *Acer saccharinum* or the *Ulmus* association as they are in the *Acer rubrum* association of the bog series. The explanation is to be found in the absence in the one case

and the presence in the other of abundant humus, and in the exposure to floods in the one case and freedom from floods in the other. Two alluvial soil inhabitants may be found in the *Ulmus* association but are not common. These are *Lepiota alluvianus* and *Hypholoma velutinum*. The floodplain *Quercus rubra* association has a small number of fungi composed chiefly of those attached to buried débris. The fungus community is quite materially affected by the size of the river and the size of its watershed. The Desplaines River valley is often flooded. If this were not so the early associations of higher plants would be less extensive, the climax association would be proportionally greater in extent, humus would be in greater quantity, and fungi would be abundant. Such is the case at Edgebrook, where the north branch of the Chicago River has formed the floodplain. In the Edgebrook *Quercus rubra* association occurs *Lactarius vietus*, *Russula obscura*, *Thelephora Schweinitzii*, *Cortinarius coloratus*, and numerous epixylous forms on débris and decaying stumps. In the forest at Olympia Fields the connection of the forest with the topography of the small stream flowing through it is more difficult to establish. The *Quercus rubra* association is very rich in fungi, and the association of fungi seems to belong to the ravine series. A comparison of the two hydrarch successions shows the following comparative points:

	Bog hydrarch	River hydrarch
Humus supply	Abundant	Not abundant
Temperature	Favorable	Favorable
Moisture	Abundant	Abundant
Aeration	Good	Poor to medium
Alternation of flood and non-flood conditions	No	Yes
Removal of the accumulated humus	None or little	Much

The fen is represented in several localities in this area. Long Lake south of the dunes is bounded by fens sufficiently filled in for the growth of fungi. *Calamagrostis inexpansa* is the predominating grass. Humus composed of decaying grass and débris is present. A community composed of *Omphalia onisca*, *Clitocybe laccata*, *Pholiota mycenoides* (on moss), *Mutinus caninus* and on the débris *Pholiota unicolor* and *Pholiota marginata* is here present. Quite extensive fens

occur about half a mile from Lake Michigan north of Waukegan. As at Long Lake, no fungi occur where the water is constantly standing, but at the margin where *Calamagrostis inexpansa* occurs is the community with the species named. Another zone occurs here which has a community of *Hygrophorus miniatus*, *H. conicus*, *H. chlorophanus*, and *Leptonia asprella*. It is difficult to trace the succession any further in the Chicago area without an extensive study of the species found on the prairies. To make this study would require very careful observation, as the species on the prairie are doubtless few and far between. Fens have two possible developments: they may eventually develop into prairies or into the swamp forest.

The rock series is best represented near Lemont. By far the most important fungi in the early stages of this series are the lichens. These have been discussed by CALKINS and FINK. They occur in the rock succession in the order crustose, foliose, and fruticose. Such a habitat would not support fungi were it not for the peculiar structure of the lichen which makes nutrition possible by photosynthesis. Such species as *Placodium cinnabarinum* and *Lecanora hageni* are common crustose lichens. *Physcia stellaris*, *Parmelia cetrata*, and *Dermatocarpon miniatum* are foliose lichens common on these rocks, while in the chinks and on ledges *Cladonia*, a fruticose lichen, is present. *Urnula craterium* occurs in the open forest of the rocky topography at Devils Lake, Wisconsin, and in the Illinois Ozarks. Other fungi found in this series in the succeeding associations are related to the accumulation of humus rather than to the underlying rock.

The dunes present a series of higher plant associations constituting a xerarch succession. Many of these are inhabited by fungus communities. The higher plant associations may be divided for this discussion into the middle beach with such annuals as *Cakile edentula;* the upper beach with the fore dune beginning its development around the grasses *Ammophila arenaria* and *Calamovilfa longifolia;* the *Pinus* association; the *Quercus velutina* association; the *Q. rubra* association; and the climax forest association. In the upper beach grass association is found a community of fungi composed of *Psilocybe arenulina* and *P. ammophila*. These have their vegetative growth in the sand, which seems to be barren of organic material so

far as can be determined by casual observation, but the presence of the fungi indicates that some organic material is present. By sliding the hand carefully below *Psilocybe arenulina* and lifting it, sand and all, it may then be gently lowered into a bucket of water. The mycelium floats outward, and its diameter may be about 3 inches. Sand particles cling to the hyphae. At least a film of organic material must be present on the sand particles. Near the roots of *Ammophila* and *Calamovilfa* may sometimes be found *Ithyphallus impudicus* and *Coprinus atramentarius*, and as these are generally associated with conditions created by man, their appearance here is doubtless subsequent to the advent of man.

The *Pinus* association is of three types: the young *Pinus* stage; *Pinus Banksiana;* and the very old *Pinus Strobus* association. The young *P. Strobus* association is inhabited by a community of fungi composed of *Clavaria muscoides, Clitocybe pinophila*, and *Lepiota cristata*. *Boletus americanus* sometimes enters this association, and occasionally *Lepiota cinnabarinus*.[1] The pine needle carpet becomes a mass of mycelium beneath the surface by the beginning of October. The surface needles alone appear to be unaffected by the enzymatic action of the mycelium. The action of fungi on the vegetable material of the forest floor breaks down the complex organic compounds into simpler ones. The soil by repeated deposition of leaves increases in humus supply. The young pine association contains but few species of fungi, but an old pine association such as occurs at the southern edge of the dunes at Furnessville, Indiana, contains many. The *Pinus Banksiana* association differs from the *P. Strobus* association in late October by the entrance of *Boletus granulatus*. The old associations of this tree contain many specimens of *B. americanus*, and occasionally *B. chrysenteron*.

The *Pinus Strobus* association at Furnessville, south of the dunes, contains trees measuring upward of 15 inches in diameter. The vegetable material on the soil is several times as thick as it is in the young pine association. The species of fungi may be more than a hundred, of which the dominating ones only are listed here. Very numerous on the forest floor, but usually overlooked because of small size, are

[1] This species is not listed by KAUFFMAN, but is well pictured in COOKE's volumes of colored plates.

Mycena vulgaris and *Marasmius androsaceus*. *Polyporus Schweinitzii*, shown in fig. 4, attaches to the pine roots. This figure also indicates the large size of the pine trees. Other species present in this association are *Clitocybe cyathiforme*, *Inocybe destricta*, *Tricholoma equestre*, *Craterellus dubius*, *Russula decolorans*, *Lactarius lignyotus*, *Hydnum nigrum*, *Clitocybe catina*, *Tricholoma panaeolum* var. *caespitosum*, *Cortinarius semisanguineus*, *Russula chamaeleontina*, *Helvella crispa*, and *Clavaria flava*.

FIG. 4.—*Polyporus Schweinitzii* in old pine association; size of trunk of *Pinus Strobus* in background indicates age of association; dark brown fungus grows attached to pine roots.

Coleybia butyracea, which occurs here also, appears in the *Juniperus virginiana* association of southern Ohio. It may be expected that plants dependent for nutrition on the organic material furnished by the higher plant association in which they occur, may sometimes occur in two associations, if these furnish organic material of similar chemical composition. This species continues to appear where the *Juniperus* has been replaced by *Quercus velutina*, indicating the persistence for a long time of the determining chemicals. The old pine association is the exception rather than the rule in this area. The

usual course of events initiates the *Quercus velutina* association without any great accumulation of humus under the pines. The usual association of fungi is therefore the one recorded for the young *Pinus* and the *P. Banksiana* associations.

The soil of the *Quercus velutina* association throughout the dunes, where changes are comparatively rapid, contains scanty humus. Areas become stabilized, but the protecting dune that made possible the stabilization may gradually shift, and the surface is again subject

FIG. 5.—*Amanita phalloides* in its habitat in the *Quercus velutina* association.

to wind action. The fungus community in this habitat composed of *Tylostoma campestris*, *Geaster hygrometricus*, *G. delicatus*, *Scleroderma flavidum*, and *Polyporus cinnamomeus* grows on scanty humus. The *Quercus velutina* association with considerable accumulation of humus is common in the protected areas, where leaves form a thin covering on the forest floor, so that the sand is not so bare as in the preceding habitat. The community consists of *Amanita phalloides* shown in fig. 5, *Hydnum zonatum*, *Hygrophorus virgineus*, *Tricholoma acre*, *T. transmutans* (KAUFFMAN states that this species forms mycorhiza on the roots of *Quercus velutina*), *T. terriferum*, *Clitocybe ochropurpurea*, *T. personatum*, *Boletus affinis*, *B. felleus*, and *B. edulis*.

The next association of higher plants contains a fungus community composed of many species. The soil of the *Quercus rubra* association is carpeted with a leaf covering thick enough to supply organic material for the development of numerous fungi. Added to the favorable supply of humus is a favorable amount of moisture retained in the soil by the protective leaf covering. The soil, as previously explained, retains its temperature late into the autumn. The number of fungi when the season is rainy is tremendous. Among these may be enumerated *Omphalia gracillima*, *O. albidula*, *Clitocybe maxima*, *C. caespitosa*, *Entoloma jubatum*, *Hydnum aurantiacum*, *H. repandum*, *Cortinarius Atkinsonianus*, *Geaster rufescens*, and *Inocybe geophila*. In this same association, and occurring in protected ravines with the *Acer saccharum* association, are *Geaster triplex*, *G. saccatus*, *Clavaria cristata*, *Clitocybe candicans*, *C. claviceps*, *Amanita flavoconia*, *A. bisporiger*, *Russula virescens*, *Boletus separans*, *Psalliota abruptibulba*, *Hygrophorus flavodiscus*, *Lactarius insulsus*, *Russula mariae*, and *Hypholoma incertum* var. *sylvestris*.

The most common climax association of this area contains *Acer saccharum* as the predominating tree species. Such an association is nearly unmixed with other trees in the ravines at Lisle. Observations are not sufficiently complete for the entire separation of this fungus community from that found in the *Quercus rubra* association. *Clavaria pistillaris*, *C. fragilis*, *Lepiota cristatatellus*, *L. rubrotincta*, *L. granulosa*, *L. glioderma*, and *Inocybe albodisca* belong to this ravine association. Doubtless the list should be much longer. Many fungi found in the climax forest are also found in preclimax associations. The explanation of this is to be found in the slow change of the humus material in the soil, and in the similarity of much of the leaf mould. Another consideration is the settling of the wind-carried leaves from the upland forests into the lower, protected, ravine forest. None of our *Acer saccharum* forests are extensive enough to prevent the mixing of the leaves on the forest floor.

The *Fagus grandifolia* climax forest at the eastern extremity of the area is not pure. Forests in other parts of the country indicate that *Boletus* furnishes a large number of species for this climax. Near Bainbridge, Ohio, in the ravines of the Rocky Fork canyons,

are beech forests almost unmixed with other trees. A two-hour visit here in late August, 1926, revealed a fungus flora of which nearly half of the individuals present were *Boleti*. Among these were *B. alveolatus*, *B. separans*, *B. subsanguineus*, *B. Russelli*, and others. It is stated by KAUFFMAN that certain *Boleti* form mycorhiza on the roots of *Fagus*. This may indicate either a relationship between fungi and the advance of the forest to the climax, or the necessity

FIG. 6.—*Omphalia campanella* on a stump in the Olympia Fields forest, showing one of the species of fungi responsible for breaking down of complex to simple organic compounds, reduction of wood to humus.

of the presence of the *Fagus* rootlets for the development of the *Boleti*.

In the foregoing discussion, the terrestrial fungi only have been included in the associations. The chief interest of the epixylous species within the scope of this paper is in that they reduce the woody material to soil humus. Fig. 6 shows a stump in the forest being reduced by enzymatic action of the fungus *Omphalia campanella*. Hundreds of epixylous species are at work decaying stems, logs, and woody débris. A discussion of these should be contained in a separate article.

The nomenclature used for the higher plants is taken from Gray's *New manual of botany*, seventh edition. The nomenclature of the Agaricaceae is the same as that in *The Agaricaceae of Michigan*, by KAUFFMAN. The nomenclature of *Boletus* is that of PECK; The nomenclature of the Polyporaceae is the same as that contained in *The Polyporaceae of the middle-western United States*, by OVERHOLTS. In the Hydnaceae, Thelephoraceae, Clavariaceae, and Gastromycetes the nomenclature is the same as contained in *The higher fungi of the Chicago region*, by MOFFATT.

The writer is under obligation to Professor HENRY C. COWLES, of the University of Chicago, for help and inspiration in this work, and to Dr. W. B. MCDOUGALL, of the University of Illinois, for his critical examination of the material contained in this article.

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ORIGIN AND DEVELOPMENT OF TISSUES IN RHIZOME OF PTERIS AQUILINA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 368

C. Y. CHANG

(WITH EIGHTEEN FIGURES)

Introduction

The aim of the investigation here reported was to determine as accurately as possible the origin of tissues, and to trace their development to maturity. The need of anatomical studies of this nature the writer undertakes to demonstrate in the following pages.

The rhizome of *Pteris aquilina* was chosen for various reasons. It represents a class of stems that are creeping and subterranean, with peculiar problems of their own. The various structures of the stem stand out distinctly from one another, enabling one to trace them to their starting points with considerable certainty. The derivation of all cells, and consequently of all tissues from a single apical cell, brings the investigator closer to the common source of various tissues than is possible with a generalized meristem. Moreover, the availability of material in any desired quantity eliminates the danger of generalization on insufficient data.

The investigation deals first with the gross anatomy of the rhizome, then with the apical cell and its derivatives, the origin and development of endodermis, pericycle, phloem, and xylem, and finally the origin of adventitious roots.

Materials and methods

Stem tips of *Pteris aquilina* were collected in the sand dune region near Chicago. Collections were made mostly in June and early July, when the growth of the rhizome is most vigorous. As regards the field conditions of this most common fern, there is little to add to the extensive observations of HOFMEISTER (**2**), save the fact that the rhizome may be very deeply buried, sometimes 6–8 inches below the surface of the soil.

Gross anatomy

The internal structure of the rhizome is truly dorsiventral. A cross-section reveals the peripheral bundles on the lateral and ventral sides as small circles and ovals. Dorsally the peripheral bundle assumes the form of a band, frequently as long as the two central bundles. The two sclerenchyma bands are also decidedly different.

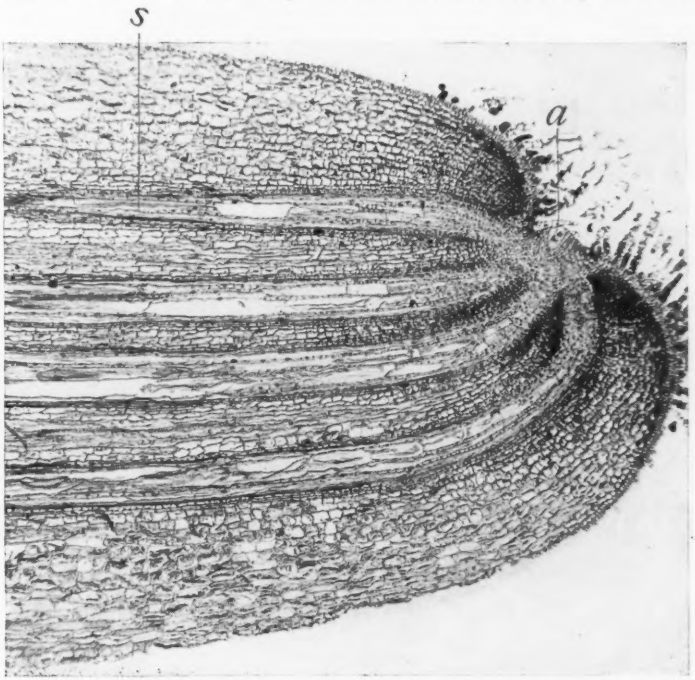


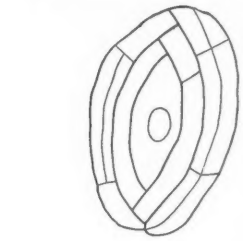
FIG. 1.—Longitudinal dorsiventral section of rhizome: *a*, apical cell; *s*, cells that will mature into sclerenchyma; ×48.

The lower band is longer and curves upward, while the upper one is the shorter of the two, and usually straight. As a rule the lateral ridges are situated dorsally to the median plane. In making sections these features were utilized to orient the material imbedded in paraffin, and it was found that this dorsiventral arrangement held true in every case.

At the tip the bundles converge and meet behind the apex (fig. 1).

As the apical cell is situated dorsal to the median plane, the ventral peripheral bundles describe a large curve to come to the meeting point; hence at any given transverse plane of the rhizome, the ventral peripheral bundles are the oldest in the developmental history. The dorsal central bundle or the one above it (the dorsal peripheral bundle) may run practically straight to the apex, and consequently either may be the youngest in the development in a given transverse section.

The secondary roots are connected with the peripheral bundles on the ventral and lateral sides. The central bundles have no root connections.



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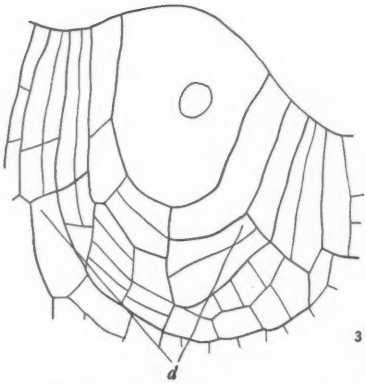

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FIGS. 2, 3.—Fig. 2, transverse section of apical cell; fig. 3, longitudinal section of apical cells; ×290.

Apical cell and derivatives

The apical cell of the stem, unlike that of the root, does not terminate the geometric axis of the stem, but is dorsally situated and turned upward (fig. 1*a*). The longest diameter of the apical cell makes an oblique angle with the axis of the stem. Of fourteen stem tips measured, this angle varies from 40–79°, the average being 62.5°.

The best view of the shape of the apical cell is to be obtained from transverse sections of the cell. To obtain this view the stem tip has to be mounted at such an angle that the microtome knife is most likely to strike the cell perpendicular to its longest diameter. Since this diameter, as already pointed out, makes a varying angle with the geometric axis of the stem, sections perpendicular to it were obtained only after a large number of trials. In this view the apical

cell has the shape of a double convex lens, with the longer diameter parallel to the axis of the stem (fig. 2). It has two cutting faces, therefore, giving off concave-convex segments alternately right and left. As the apical cell with its immediate segments protrudes above the neighboring surface, the cross-section severs it from the adjacent tissues. The lateral view of this cell, a view parallel to the dorsi-ventral plane of the rhizome, shows it to be somewhat obovate in outline, with a broad curved free surface and a deep cup-shaped inner surface (fig. 3). It amounts to a modified form of the dolabrate apical cell that prevails in the anacrogynous Jungermanniaceae. Deviations approaching the triangular-pyramidal type occur.[1]

Both anticlinal and periclinal divisions take place in the second segment of the apical cell. The outer half of the products of the periclinal division remains parenchymatous. It is from the inner half of the segment that desmogen strands arise (fig. 3 *d*). These are distinct from the surrounding tissue 8–10 cells below the apical cell (fig. 1). The tissue intervening between the peripheral and central bundles remains parenchymatous for a time, but 1 mm. below the apex most of its cells elongate and become prosenchymatous (fig. 1 *s*). Finally their cell walls become thickened and pitted, and the tissue matures into the sclerenchyma.

Five or six segments away from the apical cells, periclinal divisions of the peripheral cells suddenly increase, especially on the dorsal and lateral sides (fig. 1). As a result, an inclosure is built around the apex. The mode of formation of this pit is described at length by HOFMEISTER.

Endodermis

Long before the endodermis cells are filled with a dark staining substance, they are easily recognized by the Casparian strips which line the radial and cross-walls of the endodermis cell (fig. 16 *ca*). These strips can be traced up the stem to within 0.7 cm. of the tip. In sections stained with safranin and light green the strip is recognizable at first as a green, more or less vacuolated band, but one or two cells down the green stain gradually gives place to the red, indicating the beginning of deposition of an impregnating material

[1] Cf. KLEIN (5), Pl. IX, figs. 23, 29, 30, 31.

which is regarded by PRIESTLEY and RADCLIFFE (**8**) as of a fatty acid nature.

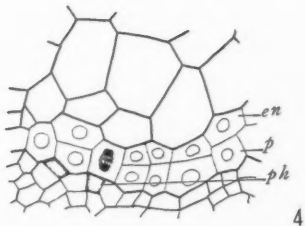
The endodermis and pericycle cells, which even in quite young tissue are readily distinguished by the shorter and denser cells of the latter, become indistinguishable in the embryonic region. This immediately suggests the probability that the cells of both layers are descendants of a common mother cell. Absolute proof of their common origin is not to be obtained, however, until mitotic figures are studied. These were observed in both longitudinal and transverse sections, and establish the fact that one cell divides periclinally, giving rise to the endodermis cell on the outside and pericycle cell on the inside (figs. 4, 5).

Although it is beyond the scope of the present paper to deal with the function of structures, it was noted that when the tissue underwent plasmolysis, the protoplast of the endodermis cell withdrew from all parts of the wall except from the Casparian ring. There the protoplast seemed to be "cemented" to the ring (fig. 6). This is in accordance with the claim of PRIESTLEY and NORTH (**7**) that water and solutes have to pass through the protoplast of the endodermis cell, and shows also that the semipermeable nature of the endodermis is not affected by plasmolysis. The air spaces between cortical cells are significant and will be discussed later.

Pericycle

The mature pericycle consists mainly of a single layer of cells, although in places doubled. As a rule these cells have a greater radial diameter than those of the endodermis. Beyond the meristematic region, where the cells of both layers are alike, the pericycle cells are shorter. This evidently is due to the fact that the pericycle cell has greater capacity for division and retains it longer. Sometimes the divisions are so frequent that the pericycle cells are isodiametric, while the endodermis cells are always elongate (fig. 7); but 0.5 cm. from the tip pericycle cells also cease dividing. The subsequent growth of the pericycle is entirely through elongation.

The origin of the pericycle has been treated in connection with that of the endodermis. The second layer of the pericycle, when there is any, ordinarily arises from the subsequent periclinal divi-



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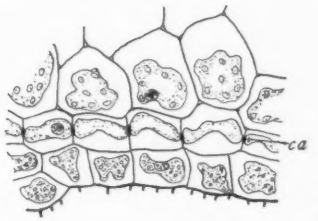

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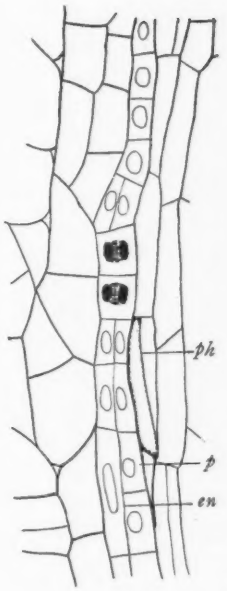

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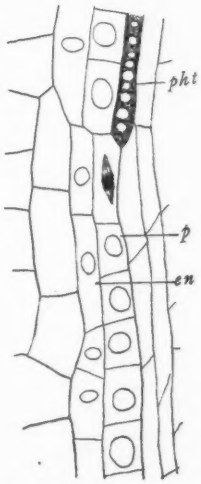

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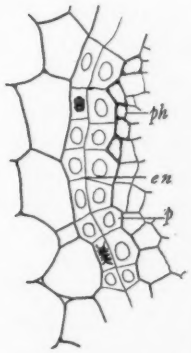

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FIGS. 4–8.—Fig. 4, transverse section of rhizome, showing common origin of endodermis and pericycle (*en*, endodermis; *p*, pericycle; *ph*, protophloem sieve tube); fig. 5, longitudinal section showing same; fig. 6, transverse section through plasmolyzed tissue (*ca*, Casparian strip); fig. 7, longitudinal section showing relative length of endodermis and pericycle cells (*en*, endodermis; *p*, pericycle; *pht*, piece of protophloem sieve tube); fig. 8, transverse section a little later than fig. 4; ×430.

sion of the first, but it was observed that the endodermal layer in the embryonic region may also divide once periclinally (fig. 8). As the mature endodermis observed is always uniseriate, it follows that the inner of the two daughter cells eventually becomes part of the pericycle, the outer one alone remaining endodermal.

The common origin of the endodermis and the pericycle naturally raises the question as to whether these two layers are cortical or stelar. If their common origin occurred very early, before the embryonic tissue differentiates into periblem and plerome, the question would always remain an open one, but such is not the case in the stem of *Pteris aquilina*. From the products of the apical cell plerome is very early distinguishable from periblem, by virtue of the denser contents and hence the deeper staining quality of the latter (fig. 1). The mother cell of the two layers is unmistakably on the stelar side. Further evidence is furnished by their kinship with cells of the protophloem. In both transverse and longitudinal sections, the mother cells of the endodermis and the pericycle on the one hand, and those of the protophloem on the other, were traced again to a common mother cell (figs. 9, 10); thus no room is left for doubt of the stelar origin of both the endodermis and the pericycle.

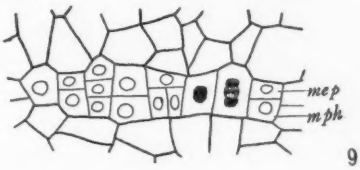
Phloem

PROTOPHLOEM.—Abutting on the pericycle on the inside is a uniseriate layer of protophloem sieve tubes, interrupted at frequent intervals by phloem parenchyma, and at the corners of large bundles often by xylem elements. In the cross-sectional view of the mature rhizome, the identity of these tiny tubes is obscured by the appearance of the parenchyma cells, which at this stage also possess thick walls and are often depleted of cell contents. In the developmental stage, however, the protophloem sieve tubes stand out from the thin walled, deep staining cells of the parenchyma.

The thickenings on the wall of the protophloem sieve tubes appear early. In transverse sections 15 μ thick the tubes begin to show secondary deposition on their walls at the fortieth section, or 0.6 mm. from the extreme tip,[2] when these cells are still dense with

[2] Descriptions here as in following cases are based on observations of large bundles with more or less straight courses. The differentiation of the lower peripheral bundles appears earlier in a given cross-section, as has been pointed out under Gross anatomy.

contents (figs. 4, 16). Above this point the cells of sieve tube destiny are indistinguishable from the neighboring phloem parenchyma cells.



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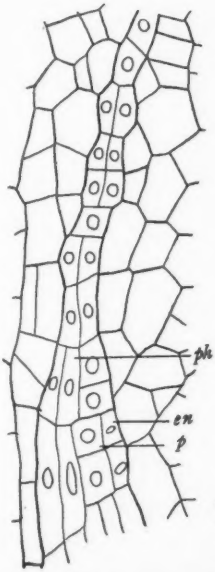

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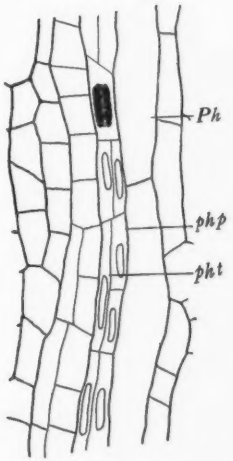

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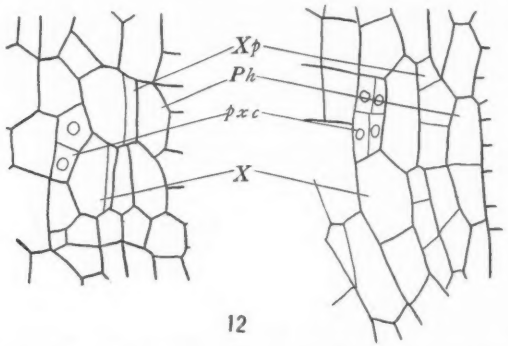
FIGS. 9–11.—Fig. 9, transverse section showing common origin of endodermis, pericycle, and protophloem (*mep*, mother cell of endodermis and pericycle; *mph*, mother cell of protophloem); fig. 10, longitudinal section showing same feature (*en*, endodermis; *p*, pericycle; *ph*, protophloem); fig. 11, longitudinal section, showing common origin of protophloem sieve tubes and phloem parenchyma (*pht*, sieve tube cell of protophloem; *php*, parenchyma cell; *Ph*, metaphloem cell); ×430.

In fact, as has been implied in connection with the description of the endodermis and pericycle, the sieve tubes and parenchyma cells are derived from a common layer of cells. A large number of cases was

observed where the mother cell was caught in the act of division, giving rise to two daughter cells, the outer one of which matures into a sieve tube, while the inner one remains parenchymatous (fig. 11). The common origin of the endodermis and pericycle and of the protophloem has already been described.

METAPHLOEM.—The metaphloem consists of sieve tubes only. In a number of cases the differentiation of metaphloem sieve tubes is scarcely behind that of the protophloem. At all events, the meta-



FIGS. 12, 13.—Fig. 12, transverse section 0.5 mm. from extreme tip (*pxc*, cells that produce protoxylem tracheids and parenchyma cells around them; *X*, metaxylem cell; *xp*, mother cell for a group of wood parenchyma cells; *Ph*, metaphloem cell); fig. 13, transverse section next to fig. 12; ×430.

phloem develops remarkably early. Less than 0.5 mm. from the tip, a ring of cells inside of and next to the protophloem can be singled out as of metaphloem destiny by reason of their comparatively large caliber (figs. 12, 13 *Ph*), and as early as three or four cells below that, faint thickenings begin to appear as fine meshes on the walls (fig. 16). Localized sieve plates appear much later. Observations have led to the belief that these sieve areas are left as islands of thin places with tiny perforations, when the rest of the wall has been uniformly thickened with secondary deposition (fig. 16).

Xylem

PROTOXYLEM.—Each of the small bundles has one centrally placed protoxylem strand. The larger bundle as a rule has two

strands well spaced in the middle. Only when one bundle anastomoses with its neighbor may protoxylem be absent from it, although sometimes at some level a strand may consist of one or two elements only. Spiral thickenings begin to be laid down within 1 mm. of the tip. Traced from this point toward the apex, protoxylem cells can be seen as a group of elongate cells of small caliber, but here they cannot be distinguished from the surrounding cells of wood parenchyma. We have here a case parallel to that of protophloem development. The tracheids of protoxylem and parenchyma cells encircling it are close cousins. Their common ancestry can be traced to two cells (fig. 12), which divide into four (fig. 13). Irregular divisions then follow and several cells in the center of the group mature into tracheids (fig. 14). In development the protoxylem lags behind the protophloem in all bundles except the lower peripheral ones (fig. 15); there the protoxylem is a little ahead of the protophloem.

METAXYLEM.—Metaxylem cells have the curious feature of being the earliest group to differentiate and the last to mature. As early as twelve cells below the apical cell, those of metaxylem destiny are distinguishable by their larger lumina and rarefied contents, but as a rule the earliest secondary thickening cannot be detected until 0.5 cm. from this point (fig. 16). The wall thickenings of the metaxylem are laid down only after cell elongation has ceased.

Unlike the protoxylem cell, which acquires the initial cambiform shape through longitudinal divisions after the cessation of mitosis in the transverse plane, the metaxylem element gains its form entirely through elongation. Its mitotic activity ceases while still in the isodiametric form. It lengthens as its neighbors divide transversely, but the greatest elongation occurs with the general elongation of all cells, which in a vigorously growing rhizome takes place about 0.5 cm. from the tip. At this point the length of the cortical cells averages 120 μ as compared with an average length of 14 μ of the corresponding cells of the embryonic region. This is an increase of eight times. The metaxylem cell whose length subtends three or four cortical cells at the lower meristematic region is lengthened correspondingly. A general rule of cell elongation may be formulated. The cell which ceases division earliest in a given plane will be the longest cell in the direction perpendicular to that plane. For in-

stance, the cell which ceases division transversely two generations ahead of a cell at its right will be four times as long as the average of the granddaughter cells of its one time neighbor, whatever length

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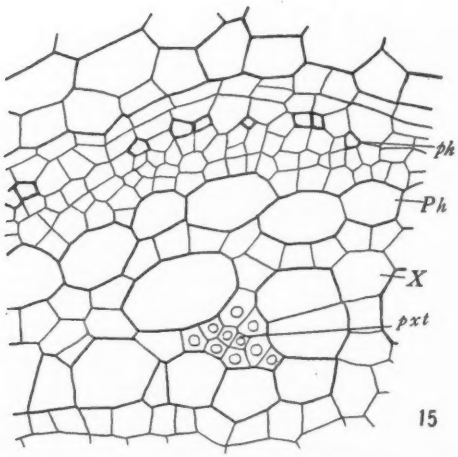


FIGS. 14, 15.—Fig. 14, transverse section showing protoxylem; fig. 15, transverse section showing earlier development of protophloem than protoxylem (*pht*, sieve tube of protophloem; *px*, protoxylem cells; *Ph*, metaphloem cell; *X*, metaxylem cell); × 430.

the latter cells may attain. Exceptions must be made, of course, of cells haustorial in nature and of those which gain length by sliding past others. The cells that give rise to tracheids and sieve tubes cease to divide earlier in the transverse plane than the rest, and hence their greater length in the cell community.

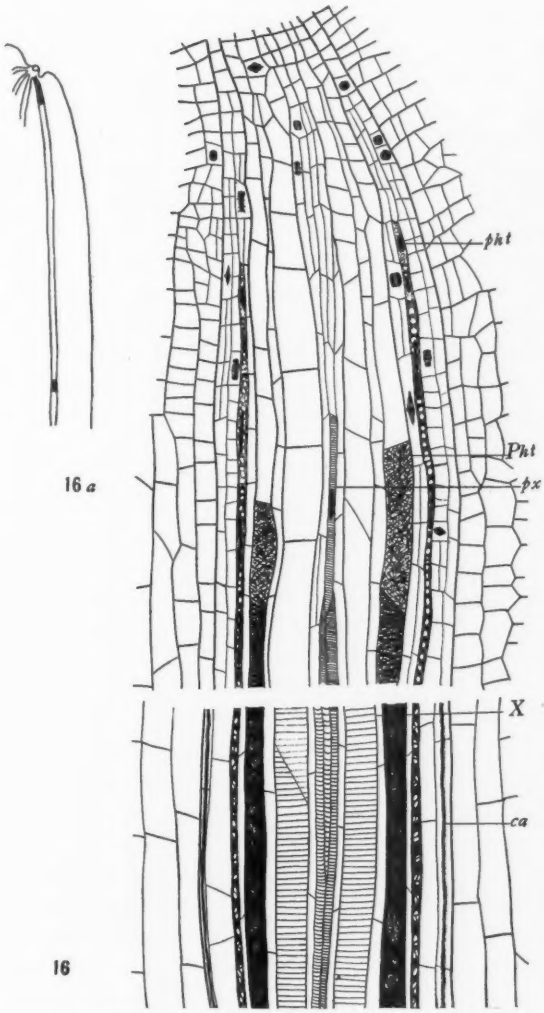


FIG. 16.—Reconstruction of bundle (*pht*, protophloem sieve tube; *Pht*, metaphloem sieve tube; *px*, protoxylem tracheid; *X*, tracheal segment of metaxylem; *ca*, Casparian strip of endodermis); hiatus represents an omission of 0.46 cm. of actual length, or 6.10 cm. on magnified scale; *a*, longitudinal section showing relative position of the two parts of fig. 16; ×134.

WOOD PARENCHYMA.—The origin of wood parenchyma cells surrounding the protoxylem has already been described. The origin of the rest of wood parenchyma has not been thoroughly investigated. Cases were observed, however, which indicate that groups of parenchyma cells are descended each from a mother cell, and this cell is a sister of the neighboring metaxylem cell. Figs. 12 and 13, drawn from consecutive sections, show this mode of origin of cells of wood parenchyma clearly.

Adventitious root

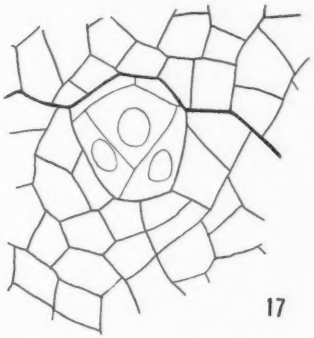
The adventitious roots appear extremely early. Cases were noted where a secondary root had reached a length of 1 cm. when it was only 2 mm. from the tip. The roots are connected with the small peripheral bundles on the ventral and lateral sides. When the small bundle begins to assume definite form, the large triangular-pyramidal apical cell of the root is already conspicuous with a number of segments cut off. It is clearly recognizable soon after the meristem is differentiated into periblem and plerome. This apical cell originates in the outermost layer of the plerome, but not infrequently the assignment requires careful study (fig. 17). It is obvious that the triangular-pyramidal cell is derived from an isodiametric one. The mode of producing a four-sided cell from a six-sided one is the same as in the Bryophytes. The outline of the original isodiametric cell generally remains distinct after a considerable number of subsequent divisions (fig. 18).

Discussion

The present findings on the shape of the apical cell of the rhizome are largely in accordance with the observations of KLEIN, so far as he has gone. Of the fifty species of ferns with creeping habit which KLEIN examined, he finds *Pteris aquilina* the only one that possesses an apical cell with two cutting faces "zweischneidig," the rest all having the triangular-pyramidal type. He concludes, therefore, that the form of the apical cell has no significance in relation to dorsiventrality. Still there are differences between *Pteris aquilina* on the one hand and the rest of the ferns with creeping stems on the other. To my knowledge, *Pteris* is the only one that is deeply penetrating; the other creeping forms are either trailing or very loosely covered with soil. Also *Pteris* is the only one with a dorsiventral structure. Thus

there is a striking coincidence (which may prove a correlation) between the "zweischneidig" apical cell and the truly dorsiventral rhizome, a difference which should not be ignored.

In his observations the writer has found nothing that is out of harmony with the opinion of LAND[3] that the apical arrangement of *Pteris* is a case of admirable adaptation. The growing point is turned upward, presenting the older and firmer tissue to the pressure of the forward thrust. It is located at the bottom of a pit, which protects it from any abrasion as the rhizome elongates, and the delicate point



FIGS. 17, 18.—Fig. 17, section parallel to transverse view of apical cell of rhizome, showing apical cell of root just after root formation; heavy line marks boundary between periblem and plerome; fig. 18, later view of apical cell of root; ×430.

is further protected by a very dense covering of hairy scales. This opinion is strengthened by the fact that in *Polypodium aureum* and *P. vulgare*, which are creeping but superficial, the apical cell terminates the geometric axis of the stem.

In his work on the anatomy of the leaves and stems of ferns, RUSSOW (**9**) observed the similarity in shape and size of the juvenile cells of the endodermis and the pericycle, and concluded that there was a common origin for these two layers in *Pteris aquilina* and other forms. The unreliability of conclusions drawn from the appearance of cell shape, however, is illustrated by the fact that VAN TIEGHEM and DOULIOT (**13**) in their work claim a separate origin for these two layers in *Pteris*. That no importance has been attached to RUSSOW'S

[3] Private communication.

report was shown by a statement of JEFFREY (4), who, in describing the bundle of *Pteris aquilina*, states:

> Within the endodermis is situated a layer one or sometimes two cells in breadth, the pericycle, which constitutes the external boundary of the fibro-vascular tissues, just as the endodermis marks the internal limit of the fundamental system.

The similarity in appearance of cell shape that led RUSSOW to believe in the common origin of the endodermis and pericycle, also led him to conclude that both these layers were derived from the ground tissue, a conclusion which the writer's findings contradict.

The astonishing fact, however, is that while the statement that the endodermis constitutes the innermost layer of cortex is found almost universally in botanical textbooks, no conclusive account based on actual observation has been found, which shows this mode of origin of the endodermis. VAN TIEGHEM'S positive statements were not supported by illustrations (12), and were contradicted by reports of his contemporaries (3, 6). Concerning the figures by VAN TIEGHEM and DOULIOT (13), a number of which support VAN TIEGHEM'S claim, SCHOUTE (10) states:

> Die gegebenen Figuren, von VAN TIEGHEM und DOULIOT nach aus freier Hand angefertigten Schnitten gezeichnet, sind aber sehr schematisch.

The comparatively recent work on the origin of the endodermis was on *Hippuris*, investigated by SCHOUTE (10), and later confirmed by BARRATT (1). They found that the endodermis and several layers outside of it were derived from the plerome.

If with further investigation it proves to be generally true that the endodermis is a part of the stele, then any theory on structural evolution which bases the main weight of its argument on the endodermis being cortical will necessarily require reconsideration.

The common origin of protophloem sieve tubes and phloem parenchyma makes the latter a part of protophloem. The fact that from a homogeneous group of cells, only the outer ring is transformed into sieve tubes, is probably not without significance. A parallel case is the development of endodermis and pericycle. Starting from two

sister cells exactly alike in all respects but position, the endodermis cell undergoes pronounced transformation through acquiring a Casparian ring, and later a suberized wall (8), while the pericycle cell remains unchanged. A still more remarkable fact is that when a juvenile endodermis cell divides tangentially, the inner one of the two cells remains simple and eventually forms a part of the pericycle; the outer one alone acquires the characteristics of an endodermis cell. It will be remembered that the cortex is filled with air spaces up to the endodermis (fig. 7). PRIESTLEY and NORTH point out that suberin formation is always dependent upon the presence of oxygen. It is the position with respect to oxygen supply, therefore, that determines the formation of structures characteristic of the endodermis cell. It is not without reason that one expects that the differentiation of the outer layer of protophloem into sieve tubes may also be due to its relative position, although the determining factor is not yet known.

The comparatively greater age of phloem in *Pteris* rhizome does not agree with the findings of TURNER (11) in the stem of *Lycopodium lucidulum*. In that form TURNER finds that the development of xylem is ahead of phloem. This apparent disparity is readily explained, however, when one compares the habit of the two stems: the stem of *Lycopodium lucidulum* is erect and aerial, while that of *Pteris aquilina* is creeping and subterranean. The developing stem of the former is right in the photosynthetic region, but away from water and mineral nutrients, whereas exactly the reverse condition prevails at the growing tip of *Pteris* rhizome. Water transportation is more active in one, food translocation in the other. The structural development corresponds admirably with these physiological conditions.

The comparative earliness of protoxylem development in the lower peripheral bundles of *Pteris* rhizome is probably due to their communication with the adventitious roots. A close connection with an absorptive organ is expected to have a stimulating effect on the development of a conductive structure.

Concerning the origin of the adventitious root, VAN TIEGHEM and DOULIOT came to the following conclusion:

En résumé, que la tige des Fougères, soit monostelique ou polystelique, ... la racine laterale s'y fait toujours aux dépens d'une cellule de l'endoderme actuel.

Thus these authors contrast the origin of the secondary root of ferns with that of seed plants, in which they found the source of adventitious roots to be the pericycle. The present investigation shows that the secondary root of *Pteris* starts so early that it is impossible to assign it to any morphological layer, but as it originates in a cell in the outermost layer of the plerome, it cannot help involving the endodermis, which, as has been shown, is the limiting layer of the stele. In fact, when VAN TIEGHEM and DOULIOT say that the root originates in an endodermis cell, they mean in a cell among the products of which arises the endodermis. There is, however, this radical difference. VAN TIEGHEM and DOULIOT claim that the initial of the root arises from the innermost layer of the cortex, which they consider to be the seat of the endodermis, whereas the writer's preparations show that both the initial of the root and the mother cell of the endodermis originate in the outermost layer of the stele. Both are stelar, not cortical. To show the origin of secondary roots in ferns the authors in question figured the stem of *Nephrolepis davallioides* only, and one hesitates to accept their statement since they showed the apical cell only in the advanced stage, when its assignment to any histogenic region is largely a matter of conjecture.

In conclusion, it is considered that the results of the present investigation warrant the opinion that it is only through critical study of the embryonic region that we can determine with certainty the origin of tissues, and that work which was done long ago may be worth reinvestigation.

Summary

1. The structure of *Pteris* rhizome is unmistakably and invariably dorsiventral.

2. In shape the apical cell of the rhizome is a modified form of the dolabrate type.

3. The apical arrangement is considered to be adaptive to the subterranean habit.

4. The endodermis and pericycle have a common origin and both layers are stelar.

5. The protophloem sieve tubes and the phloem parenchyma are derived from a common mother cell, which in turn has a common origin with the mother cell of the endodermis and the pericycle.

6. Protoxylem, metaxylem, wood parenchyma, and metaphloem were traced also, so far as the identity of their cells was recognizable.

7. The development of xylem lags behind that of phloem in all except the lower peripheral bundles. This difference in development is considered to be due to the subterranean habit of the rhizome.

8. The adventitious root develops extremely early. Its apical cell originates in the outermost layer of the plerome soon after its differentiation from the periblem.

9. The results of the present investigation on the origin of the endodermis and adventitious root do not agree with the descriptions in the older works.

The writer takes great pleasure in acknowledging his indebtedness to Professor W. J. G. LAND, under whose direction this investigation has been conducted. Thanks are also due to Professor C. J. CHAMBERLAIN for his kindness and encouragement.

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RELATION BETWEEN FRUIT SIZE AND ABSCISSION OF YOUNG ORANGE FRUITS[1]

A. R. C. HAAS

Excessive abscission of young citrus fruits during certain periods of the year constitutes a serious loss to the citrus industry. COIT and HODGSON[2] have described the conditions under which the navel orange crop of 1917 in certain districts became practically a total loss. Among the factors considered, particular stress was placed upon the high maximum daily temperatures that accompanied dry winds. Citrus leaves are able to withdraw moisture from the fruit, and the daily water deficits brought about in this way may cause abscission, or may interfere greatly with the development of the fruit.

The present paper is concerned with the so-called "June drop" of young citrus fruits under high maximum daily temperature, and recognizes the fact that during such periods there may be a large withdrawal of moisture from the fruit by excessive leaf evaporation. The data presented here have particular reference to excessive abscission of young fruits from citrus trees that receive the best cultural care known, and the discussion has no intended application to abscission of young citrus fruits brought about by deficient nitrogen supply, unfavorable soil salinity, deficient soil moisture, etc., conditions under which abscission of the fruits is a reasonable expectation.

It has been well established[3] that citrus leaves are able to withdraw moisture from the fruit. The writer has found in a large series of unreported experiments that the upper surface of mature citrus

[1] Paper no. 156, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] COIT, J. E., and HODGSON, R. W., An investigation of the abnormal shedding of young fruits of the Washington navel orange. Univ. Calif. Publ. Agric. Sci. 3:283. 1919.

[3] BARTHOLOMEW, E. T., Internal decline of lemons. III. Water deficit in lemon fruits caused by excessive leaf evaporation. Amer. Jour. Bot. 13:102. 1926.

leaves is practically no more economical of the moisture of the leaf than is the lower surface, and that mature citrus leaves are much less economical of their moisture than are young ones, but that in contrast the young leaves of the walnut are much less economical of their moisture than are mature ones. The almost complete absence of stomatal regulation, and the large cuticular evaporation from both surfaces of mature citrus leaves, bring about water deficits in citrus foliage and fruits during periods in which high maximum temperatures prevail, and especially when such temperatures are accompanied by dry winds. The injurious effect of such water deficits may not be evident unless their action is severe and prolonged. It is rather difficult to evaluate the effect of excessive leaf evaporation upon abscission of citrus fruits, unless we also consider the evaporation from the fruit itself. Heretofore the large evaporating power of citrus leaves and their consequent withdrawal of moisture from the fruits have caused the evaporation from the fruits to be practically ignored, although in fact there is a great difference in surface evaporation between young and older fruits.

During the month of July, 1925, at the Rubidoux Tract of the Citrus Experiment Station, high maximum temperatures were reached quickly and were maintained for but very short periods. The writer was conducting large tank experiments with Valencia orange trees in soil cultures at the time, and noted the excessive shedding of young fruits of a certain size, whereas fruits of larger sizes remained firmly attached until maturity. The soil in the tanks had been kept uniformly moist during the hot weather, and the trees were protected by a solid lath windbreak from hot, drying winds. Examination of orange trees in the experimental plots of the Station also revealed excessive abscission of the smaller citrus fruits under favorable soil moisture conditions.

Young navel oranges were picked from guard rows at the Rubidoux plots, and were immediately brought into the laboratory. The fruits were air dried in a room at about 80° F. The effect of the size of the fruit upon the loss of moisture from its surface is seen in table I. The water loss is stated as the percentage of total water lost on drying to constant weight at 140°–158° F.

Table I shows that the young fruits differ considerably in their

loss of moisture, according to their size. The smaller the fruits the more rapid is the water loss, under a given set of conditions.

In a repetition of this experiment, the fruits were dried at 80° F. in desiccators containing anhydrous calcium chloride. Several large

TABLE I

PERCENTAGE WATER LOSS FROM FRUITS OF WASHINGTON NAVEL ORANGE, AIR DRIED FOR TIME INDICATED

TIME (HOURS AND MINUTES)	WATER LOSS	
	Fruits (65), diameter about 1.7 cm.	Fruits (58), diameter about 2.5 cm.
25: 0	21.38	13.93
44:30	29.56	19.67
71:30	42.14	27.87
93:10	49.69	33.60
139: 5	61.01	42.95
162:35	66.66	48.36
188: 5	71.70	53.44

desiccators were used, and a proportionate number of fruits of each size was placed in each desiccator. In every case the fruits were left standing on the stem end.

TABLE II

PERCENTAGE WATER LOSS FROM FRUITS OF WASHINGTON NAVEL ORANGE, AIR DRIED FOR TIME INDICATED

TIME (HOURS AND MINUTES)	WATER LOSS	
	Fruits (63), diameter about 15×20 mm.	Fruits (40), diameter about 35×40 mm.
23:30	6.30	2.31
66:30	14.96	6.29
93:25	18.89	8.17
114:15	21.26	10.07
161: 0	25.98	12.58
184:25	29.13	14.26
210: 0	31.50	16.14

Table II shows that young citrus fruits may lose their moisture two to three times as rapidly as larger fruits. Another lot of 63 navel oranges (diameter 15 mm. × 20 mm.) in desiccators lost 7.37 per cent of their moisture in 24 hours, while 40 navel oranges (diameter 35 mm. × 40 mm.) lost 2.15 per cent in the same period. The rind of the

smaller fruits was about 5–6 mm. in thickness, while that of the larger fruits was about 6–7 mm.

Eighty of the small navel oranges and 33 of the larger ones were dried in an oven which was regulated to 110° F., but which rose even above 120° F. once the fruit was placed in it. The smaller oranges lost 65.61 per cent of their moisture and the larger ones 54.51 per cent in 23 hours and 50 minutes. The effect of the higher drying temperatures appears to reduce somewhat the differences in the percentages of moisture lost by the oranges of different sizes.

It was of interest to find, in the case of grapefruit also, that the smaller fruits lost a considerably higher percentage of their moisture when dried at 70°–80° F. than did larger fruits (table III).

TABLE III

PERCENTAGE WATER LOSS FROM GRAPEFRUIT AIR DRIED FOR TIME INDICATED

TIME (HOURS AND MINUTES)	WATER LOSS	
	Grapefruit (93), diameter 25×30 mm.	Grapefruit (42), diameter 40–45×45–50 mm.
19:25	16.73	9.42
44: 0	30.15	19.11
67:35	41.05	27.24

The water losses from grapefruit and Valencia oranges of approximately similar size were compared, to ascertain whether grapefruit loses its moisture less readily than Valencia oranges, and thereby is enabled to remain attached in groups or clusters even to maturity. The data in table IV show that young grapefruits lose their moisture at about the same rate as young Valencia oranges of similar size.

A field test was made to determine whether the effect of high temperatures, when accompanied by a favorable soil moisture supply, decreases with an increase in the size of citrus fruits. Young Valencia oranges (diameter 18 mm.×20 mm.) were tagged on trees in the guard rows of the Rubidoux plots on June 29, 1925. On July 22, only 36 out of 113 tagged fruit still remained attached, while among the tagged fruits of slightly larger size there was practically no abscission.

The water content of large citrus fruits is proportionately not much different from that of small fruits. For example, in table I the water content of the small fruits was 74.6 per cent of the fresh weight, while that of the large fruits was 76.2 per cent; in table III the water contents are 80.4 and 80.5 per cent respectively. Consequently the greater percentage loss of water from the small than from the large orange fruits is not due to differences in the water-holding capacity of the fruits of different sizes. The possibility exists that the reduced loss of water from the large fruit may be due to

TABLE IV

COMPARATIVE PERCENTAGE WATER LOSS OF SIMILAR SIZED YOUNG GRAPEFRUITS AND VALENCIA ORANGES

TIME (HOURS AND MINUTES)	WATER LOSS IN AIR DRYING AT 70°–80° F.		WATER LOSS IN AIR DRYING AT 102°–106° F.	
	Grapefruit (70), diameter 18×21 mm.	Valencia oranges (58), diameter 18×21 mm.	Grapefruit (70), diameter 18×21 mm.	Valencia oranges (58), diameter 18×21 mm.
23:25.....	22.31	19.03	45.04	46.29
46:20.....	36.36	31.86	69.01	71.61

deposits of gums or resins in stomatal openings. The stomatal openings of small and large navel orange fruits were eliminated by peeling off the outer green portion of the rind. Under uniform exposure on trays of large mesh wire screen, the small fruits lost 48 per cent of their water in 23 hours, while the large fruits lost only 24 per cent in the same period. Obviously the greater rate of water loss from young fruits is not due to an appreciable extent to any surface mechanism that controls the loss of water through the rind. The results may be explained on the basis of the relation of the surface area of the fruit to its volume. If for the sake of convenience we assume that the shape of an orange is that of a sphere, we have to deal with the following formulae:

$$\text{area of a spherical surface} = 4\pi r^2 \qquad (1)$$

$$\text{volume of a sphere} = \tfrac{4}{3}\pi r^3 \qquad (2)$$

$$\frac{\text{surface area}}{\text{volume}} = \frac{4\pi r^2}{\frac{4}{3}\pi r^3} = \frac{3}{r} \qquad (3).$$

If in our example we assume the radius of the small sphere to be 10 mm. and that of the large sphere to be 15 mm., we have as the relation between the surface area and the volume,

$$\text{for } r = 10, \frac{\text{surface area}}{\text{volume}} = \frac{0.3}{1}$$

$$\text{and for } r = 15, \frac{\text{surface area}}{\text{volume}} = \frac{0.2}{1},$$

or the rate would be 50 per cent more rapid for the small sphere than for the large, if the rate of loss of water is governed by the surface area per unit volume. The small orange fruits would have more surface area per unit of volume than the large ones, and would consequently have a greater opportunity to lose their water.

Considering table I in this way, for the small oranges $\frac{3}{r} = \frac{3 \cdot 53}{1}$, and for the larger oranges $\frac{3}{r} = \frac{2 \cdot 4}{1}$; hence the expected rate of water loss per unit volume from the small fruit would be 1.47 times that from the larger fruit. The table shows that for a 25-hour period the rate of water loss from the smaller fruits was 1.53 times that from the larger fruits. Discrepancies are doubtless due in part to the fact that orange fruits may deviate considerably from a sphere in shape. In table IV, in which fruits of approximately the same size were taken, we find practically the same rate of water loss, again confirming the assumption.

The surface area of orange fruit in relation to the volume is no doubt a determining factor during hot weather as to the degree of severity of the so-called "June drop." The insurance against this source of fruit loss during periods of high temperatures, when the leaves require most of the available water in the trees, lies chiefly in the direction of having the fruits set at as early a date as possible in the spring, so that when hot weather begins the fruits may be of advanced size. During periods of high temperature, when the evaporation from the leaves is very rapid, the drop of small fruits may be excessive, in spite of a favorable soil moisture supply. Experimentation as to the methods of producing an early set of fruit on orange trees may prove of considerable value.

Summary

1. The percentage loss of moisture from young oranges and grapefruit decreases rapidly as the fruits increase in size. This change doubtless is due largely to the decrease in proportionate surface area as the fruit increases in diameter.

2. Observations on tagged fruits confirm the common knowledge that young citrus fruits readily undergo abscission up to a rather definite stage of size development, but only rarely thereafter.

3. It is possible that evaporation rate is an important factor in determining susceptibility to abscission.

4. Stomatal regulation or the nature of the rind is not an important factor in determining the rate of water loss from detached fruits of different sizes.

5. The size reached by grapefruit before the time of extremely high temperatures may determine to a large extent the occurrence of fruit clusters. Detached citrus fruits of approximately the same size lose their moisture at approximately similar rates.

6. The moisture content is very similar in young orange fruits of different sizes.

7. Any climatic conditions, fertilization program, or cultural operations that may hasten the sizing of the young fruits, may give them a better opportunity to remain attached to the tree when high daily maximum temperatures are experienced.

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BRIEFER ARTICLES

THE AUXIMONE THEORY

The work of BOTTOMLEY and his pupils on the subject of plant growth-promoting substances, or "auximones," has recently been the subject of some criticism in this journal at the hands of WOLFE (10). As the only pupil concerned, and one concerned very largely with the work of BOTTOMLEY as well as with independent work, the writer cannot agree that the last word has been said on the subject, in spite of WOLFE's suggestion that the term "auximones" may well be dropped from the literature.

It is fairly evident that it is their interpretation of the experimental facts recorded in the various publications of BOTTOMLEY and the writer on the subject which is called into question, and not the accuracy of these facts. In justice to the late Professor BOTTOMLEY, however, it seems advisable that it should be made clear that it was only after his original experiments with *Lemna minor*, carried out in his own laboratory, had been repeated on a greatly extended scale in an independent laboratory open to the inspection of his botanical confrères, and the repetitions had, under these conditions, demonstrated convincingly the truth of his statements, that his first publication on work with *L. minor* appeared in 1917 (1). Since that time, the work which BOTTOMLEY and the writer have carried out, jointly and independently, has all supported the main conclusions which were then reached.

BOTTOMLEY's interpretation of the term "auximone" is brought out in his statement in 1915 (2), that "the plant food accessories resemble more closely the growth-stimulating food factors of HOPKINS than the "vitamines" of FUNK, and the term "auximone" (Gr. *αὔξιμος*, promoting growth) is suggested for them, being descriptive of their action rather than of their nature or composition." This interpretation of their rôle, as being necessary for optimum growth and development rather than for the actual maintenance of life, which was BOTTOMLEY's real intention, and which falls into line with CLARK and ROLLER's (5) conception of the accelerating action of the accessory substance for yeast, becomes apparent on careful perusal of his later work, and is supported by various statements of the writer (7).

At least one other worker has observed an increase in growth as a result of additions to mineral nutrient solutions of materials similar to those which BOTTOMLEY and the writer have used as a source of auximones. Also, since a "catalytic" effect brought about by additions of peat extract and autolyzed yeast in the hands of SAEGER (**9**) is admitted as a possibility by WOLFE, it is difficult to see why the same effect, obtained by BOTTOMLEY and the writer, and called by them a "growth-promoting" or "auximonic" effect, should be subject to criticism. The unknown nature of these substances, though, as WOLFE states, it may aggravate the difficulties, is surely no condemnation, in view of the mystery in which the nature of the "vitamines," so widely accepted as essential factors in animal metabolism, has for so long been shrouded; while the fact that certain organic substances of known constitution have, in WOLFE'S hands, failed to result in any increase in growth, is no proof that all organic substances, of known or unknown composition, will do so. The fact, reported by WOLFE, that certain organic substances, in concentrations of 50-200 p.p.m., actually depressed the rate of growth of *Lemna* plants as compared with that in mineral nutrients only, is significant as indicating the potency of small quantities of organic substances, for good or ill, in plant metabolism, although WOLFE states that the small quantities added can hardly have exercized any specific toxicity. Since precisely similar quantities of organic materials of unknown nature have been responsible for a very marked stimulation, in the experiments of BOTTOMLEY and the writer, the acceptance of a toxic effect of such materials and in such quantities as those specified presents no difficulty to the writer.

There are one or two points which need to be stressed with regard to the work of SAEGER (**9**) and MENDIOLA (**6**) which has been chiefly quoted by WOLFE (**10**). One of the most important precautions taken by BOTTOMLEY and the writer in the course of their work has been the frequent changing (not less than twice weekly) of the culture solutions used, not so much to maintain a balance of materials as to eliminate as far as possible the contamination by bacteria and algae which they found to result inevitably from a less frequent changing of solutions. Such contamination affects materially the results of the experiments, for both BOTTOMLEY (**3**) and the writer (**8**) have shown that very small quantities of the products of certain bacteria have a marked effect in stimulating growth, while certain hitherto unpublished observations on the part of the writer suggest that contamination with blue-green algae has a somewhat similar effect. In any case, it has been a matter of frequent observation that control cultures in mineral nutrients only, when left so neglected at the conclusion

of an experiment that they became visibly contaminated with bacteria and algae, improved markedly in health. Another point that BOTTOMLEY (2) has stressed is the fact that it was frequently not until after the lapse of at least 21 days that any appreciable difference in the size of the plants in the different cultures became manifest. The writer's experience goes to show that experiments lasting over a period much longer than 21 days are necessary for any sound conclusions to be drawn.

The variability in the rate of reproduction of *Lemna* rendered it important that the number of cultures for any single nutrient solution should be made sufficiently large to enable an average rate of reproduction to be obtained. In the work of the authors in question, details with regard to these points are not always given, but in some cases such details as are given show clearly that the precautions mentioned were not always observed. For instance, experiments of 23 days' duration are quoted, while SAEGER claims that he was able to grow *Spirodela polyrhiza* for 26 months in Knop/10 solution changed "at intervals usually of one or two weeks," periods which the writer has found, during more than ten years' experience with such work, to be long enough to induce contamination sufficient materially to affect the result, for it must be emphasized that relatively minute amounts of organic material have been found to bring about a marked stimulation.

The results of the experiments of SAEGER on the effect of the additions of extracts of peat and of yeast to mineral nutrient solutions support materially the conclusions of BOTTOMLEY and the writer. His comparison of the effect of an inorganic medium as compared with pond water is also in accordance with the results of BOTTOMLEY (1), although apparently he would not agree as to this interpretation of them. SAEGER'S implied suggestion, that the beneficial effect of the yeast lay in the possibility that it contained some ash constituent which supplied a deficiency in the culture solution, is negatived by the report of BOTTOMLEY on the absence of any stimulating effect due to the ash constituents of *Azotobacter* and of nucleic acid, while these materials themselves had a marked effect as growth stimulators. Similar unpublished experiments were made with the ash of yeast, with similar negative results.

Attention has been drawn to these points in view of the fact that this work, and that of CLARK and ROLLER, has been used very largely by WOLFE to support his contention that BOTTOMLEY'S theory is completely refuted, and that the advantage of the organic matter in BOTTOMLEY'S experiments lay in its restoration of the physiological balance of a solution which, it is suggested, was about ten times too concentrated. In the work

quoted, the writer sees no justification for such a claim, which appears to be based largely on a misinterpretation of BOTTOMLEY's use of the term "auximone"; but if it is indeed a fact that quantities of organic matter ranging around about 100 p. p. m. (a fair average in BOTTOMLEY'S experiments) may have such an effect in neutralizing the ill effects of such over-concentration of mineral nutrients, then BOTTOMLEY'S work has been worth while in drawing attention to such a fact.—FLORENCE A. MOCKERIDGE, *University College of Swansea, Swansea, Wales.*

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8. ———, The formation of plant growth-promoting substances by micro-organisms. Ann. Botany **38**: 723-734. 1924.
9. SAEGER, A., The growth of duckweeds in mineral nutrient solutions, with and without organic extracts. Jour. Gen. Physiol. **7**: 517-526. 1925.
10. WOLFE, H. S., The auximone question. BOT. GAZ. **81**: 228-231. 1926.

STAINING OF YEAST CELLS

Methods of staining yeast cells have been collected to 1915 by EYRE.[1] Further improvements in technique have been made by DAVIS[2] on spore staining which are applicable to yeasts, and by WINGARD[3] in his study of the nuclear phenomena of the yeasts.

With large elementary classes, the smear method, whereby a drop of the culture is evaporated on a cover slip and fixed in the flame, usually results in shrinkage and distortion of the cells, and the cell content is not readily discernible. A method which is rapid and practicable for use in large classes has been devised in the botanical laboratory of Rutgers University. This involves the same principle as that used by DAVIS, who imbedded the cells in a thin film of parlodion on a slide, but requires less time. By it there results but little shrinkage of the cell, and vacuoles and protoplasmic structures are easily defined.

The procedure is as follows. On a clean glass slip place a drop of dilute albumin fixative (Mayer's albumin, diluted 1:10 with distilled water), stir in a small drop of the yeast culture and allow to dry over gentle heat (as on a warm radiator). The material should then be stained (acid fuchsin, acting for 50 seconds gives good results), the excess washed off, then dried as before, and mounted in balsam.—ARTHUR P. KELLEY and M. B. SHOEMAKER, *Rutgers University, New Brunswick, N.J.*

[1] EYRE, J. W. H., The elements of bacteriological technique. Pp. x+518., figs. 218. Philadelphia: W. B. Saunders Co. 1915.

[2] DAVIS, W. H., Staining germinating spores. Phytopath. 12:492–494. 1922.

[3] WINGARD, S. A., Studies of the pathenogenicity, morphology and cytology of *Nematospora Phaesoli*. Torr. Bull. 52:249–290. 1925.

CURRENT LITERATURE

BOOK REVIEWS

General botany textbook

A volume[1] has recently appeared which is an amazing compendium of botanical information. To review it critically in a few paragraphs is quite impossible. One can only give some indication of its content and method of treatment.

Part I, the first 26 pages, outlines the problems of the science of botany. Part II, pages 29–297, discusses the vegetative functions of plants, and is excellently done. Part III, pages 301–554, takes up reproductive processes and life histories. Part IV reviews the great groups of seed-bearing plants, citing particularly those species of economic importance. Part V, pages 935–1012, is devoted to genetics and evolution.

The purposes of the authors, as stated in the preface, are to teach students how to think and to impart culture. "A subject has cultural value in proportion to the number of human contacts it gives the pupil, the extent to which it broadens his views and extends his interests and sympathies." The plants directly of service to man are the ones chiefly studied in the text, and attention is constantly called to the ways in which man is dependent upon them, and how profoundly they have affected the course of civilization.

Current errors in botanical instruction are noted and the misconceptions corrected, a feature that makes the book particularly valuable to the teacher whose instruction in botany was received several years ago. Thus osmotic pressure is not to be considered merely as a physical effect, produced by the bombardment of the membrane by the molecules of the liquid, but that the plant membrane acts as a solvent, and substances may pass by osmosis only when they are soluble in the membrane. Again, roots penetrate soils to a depth of three feet or more, six or eight feet when necessary to obtain water and food materials, so the practice of subsoiling, deep tillage, even soil dynamiting is unnecessary, as shown by the investigations of CHILCOTT and COLE (p. 129).

Citations of investigations are abundant, as in the case just given, and the footnotes give details of the place and date of publication. There is also a definite effort to introduce many historical references as a means of achieving the cultural aim. "The causal relationship between gravity and the direction of roots and shoots was first established by the English botanist THOMAS ANDREW KNIGHT," who devised the familiar experiment of directing growth by centrif-

[1] GAGER, C. S., General botany, with special reference to its economic aspects, and three chapters on genetics by ORLAND E. WHITE. pp. xvi+1056. P. Blakiston's Son & Co. 1926.

ugal force. A footnote gives a brief but illuminating sketch of the man. Such historical matter is found not only in footnotes but in the body of the text.

Throughout the book economic botany receives prime consideration, not because the book is intended for those students who are to become farmers or horticulturists, or who will follow some other application of economic botany professionally, but because the author conceives that the beginning student is interested best by dealing with those plants and those topics that relate botany most closely to men's needs. The author is not afraid to overstep the bounds of botany in following practical applications into related fields. Thus, under the topic Pollen and hay fever (p. 40), sensitivity to animal proteins is discussed. A patient is cited who was put to bed with hay fever in August by ragweed, but kept there until April by the feather pillows.

The book presupposes a laboratory course as an accompaniment of the text, from which "the student will get at first-hand many facts not given in the text." Certainly with such a combination a beginning student who masters both should have a good grip on the fundamentals of botany. It would make a formidable course, and the very bulk of the book may act as a deterrent to its wide adoption. It is an excellent text, however, for ambitious students.—E. R. DOWNING.

Plant life in the Alps

It is fitting that 50 years of botanical investigation, teaching, and writing, should be crowned with the production of a masterpiece. CARL SCHRÖTER began his career as a botanist almost exactly 50 years ago, and on December 19, 1925, he retired from his position of Professor of Botany in the Technischen Hochschule at Zurich, on his 70th birthday. Almost simultaneously there appeared the enlarged second edition of his great work on alpine plants.[2] It is essentially a new book, as the additions and revisions have added nearly 500 pages to the 800 which formed the edition of 1908.

In such a large and comprehensive work it is difficult to give any adequate idea of the scope and variety of its contents. Many topics are discussed and every one in an exhaustive manner. The contributions of others as well as the wide experience of the author are called upon to make these discussions broad and accurate. As an example, there may be cited the contents of a single table in which the data of not less than thirty investigators on the altitudinal limits of various types of vegetation are displayed in parallel columns. This is but a small fraction of the discussion of the vexed question of the causes and limits of alpine timberline.

Beginning with a general discussion of the distribution of the various types of vegetation in the Alps, climatic, biotic, and soil factors are considered. These different factors are closely analyzed, and their effect on various plant associations indicated. There follows a detailed discussion of the various elements comprising the alpine flora. Here will be found a series of monographs on the habits,

[2] SCHRÖTER, CARL, Das Pflanzenleben der Alpen. Eine Schilderung der Hochgebirgsflora. 2d ed. pp. vii+1288. *pls. 6. figs. 316.* Zurich: Albert Raustein. 1926.

structure, ecology, variation, and distribution of every species of importance in the entire flora. This includes not only woody and herbaceous flowering plants, but also ferns, mosses, algae, and fungi.

Special attention is given to the variations in form and structure occurring in the forest, in the subalpine and alpine scrub, in the meadows, in the alpine mats, in the fell fields, and in other characteristic communities. Structural responses to temperature, wind, moisture, light, and length of growing season are carefully considered. Each topic is so thoroughly treated that it is hard to point out special features, although the exhaustive consideration of cushion and polster forms is probably equaled in no other work.

A final section of the volume, comprising about 100 pages, is contributed by HEINRICH and MARIE BROCKMANN-JEROSCH, and covers the origin, affinities, and distribution of the Swiss alpine flora. It includes a consideration of the influence of the glacial period, and the distribution and migrations of the flora before, during, and since this glacial epoch.

The bibliography is extensive, well arranged, and brought down to include the articles in Schröter's *Festschrift*,[3] many of which relate to problems of alpine vegetation.

The entire mass of material covering the thousand closely printed pages is so well organized and so thoroughly indexed as to make reference easy. It is indeed a compendium of all matters relating to alpine vegetation, and without a rival in this particular field. It is safe to predict that it will long remain the authority on the problems connected with the vegetation of higher mountains the world over.—G. D. FULLER.

Chemistry of wood

An important pioneering piece of work has been done by HAWLEY and WISE,[4] in bringing together the data on the chemistry of wood. Their book represents the first attempt to summarize in one volume the literature on this subject.

The book is divided into five parts. Part I is the introduction. In part II is considered the chemical components of wood, including cellulose, polysaccharides, lignin, and certain extraneous components such as resins and essential oils, fixed oils, fats, and other compounds. In regard to the middle lamella of woody tissue, reference is made to the work of RITTER, who concluded that the compounds making up the middle lamella should be classed with lignin rather than with the pectins, and that the claim of botanists that the middle lamella is made up mainly of pectin or calcium pectate is not sufficiently supported by experiments. The literature on lignin is well summarized. Various formulas for lignin, which have been tentatively proposed, are given. It is pointed out that

[3] BROCKMANN-JEROSCH, H. (editor), Festschrift CARL SCHRÖTER. Veröffen. Geobot. Institute Rübel Zürich **3**: 1–36. 1925.

[4] HAWLEY, L. F., and WISE, L. E., The chemistry of wood. pp. 334. New York: Chemical Catalog Co. 1926.

none of these formulas can be considered as established, and that the term lignin cannot be applied to any definite compound present naturally in the cell wall. The various color reactions which have been used as tests for lignin are referred to, and it is shown that in the main they are probably due to an aldehyde which makes up a very small part of the lignin. A negative color test is not necessarily an indication that lignin is absent, that is, that delignification has been complete. Also, the color tests are not very specific, for some of them may be positive with natural substances, for example, oil of cloves and oil of sassafras; so the tests need to be used with caution.

Part III considers methods of analyzing wood for the various components, including cellulose, the pentosans, hexosans, and lignin, with some of the practical applications of such analyses. Part IV takes up the decomposition of wood, considering its combustion, with the mechanism of the process, the destructive distillation, the hydrolysis and delignification, and its decomposition with concentrated alkali. In regard to the hydrolysis of cellulose, cotton cellulose is used first as an example, and data are referred to, showing that even by dilute acid considerable cellulose is hydrolyzed. Part V considers wood as an industrial material, taking up certain physical properties, such as strength, specific heat, conductivity, absorption of water and other liquids, and including a consideration of decay.

The book is much more than a mere summary of the literature. The literature is critically evaluated. The present status of our information on the subject is shown, and the gaps in our present knowledge pointed out; thus further research along this line should be stimulated. The volume should be a valuable reference book for the general botanist, and an invaluable tool for the forester and technical worker in the commercial processes using wood as a raw material.—S. V. EATON.

Principles of plant growth

An elementary text on botany[5] has been published by ROBBINS, whose title suggests its major purpose. The "principles of plant growth" are presented in non-technical language, so that they may be understood by all who are interested in the growing of plants. This makes the book useful, not only as an elementary text in secondary schools, but also to all engaged in the growing of plants, as farmers, nurserymen, orchardists, etc. No attempt is made to give the structures of plants in minute detail, but the work carried on by the different parts is presented very simply and clearly. As an elementary presentation of the main activities of plants in connection with growth this text is certainly a success.

The recommended sequence of presentation is indicated by the organization of the text. It begins with an account of the living plant body and its work, and the style is very popular, presenting the general facts in such a way as to be

[5] ROBBINS, W. W., Principles of plant growth, an elementary botany. 8vo. pp. vi+299. *figs. 136*. New York: John Wiley & Sons. 1927. $2.25.

appreciated by those with no training in botany, or even in science. Following this general account of the plant body, there are chapters on absorption by the roots, food building, substances taken into the green plant and the uses made of them, movement of sap in plants, transpiration, and respiration.

After this outline of the ordinary growth activities, there are chapters dealing with the roots, stems, and pruning, flower (including pollination and fertilization), seed (including germination and seed testing), propagation, and weeds. Other chapters deal with the relation of water, temperature, and light to plant life.

All of the preceding chapters naturally deal with the activities and structures of flowering plants, as those with which plant growers are chiefly concerned. The book closes, however, with a few chapters presenting the other groups, from algae to gymnosperms, so that the reader is introduced briefly to the lower plants.

This text will certainly prove of service in extending the knowledge of botany into groups that are not reached by the ordinary texts.—J. M. C.

NOTES FOR STUDENTS

Pollen analysis and postglacial vegetation.—The various changes in the vegetation of the northern portion of the British Isles and of the Scandinavian Peninsula during postglacial times have been subjects of very considerable interest, both for geologists and botanists. As the results of extensive investigations of the peat deposits of Scotland, SAMUELSSON[6] confirmed and supported GEIKIE's conclusions that at two separate periods there had been more extensive forests than at the present time, indicating two periods of milder climate separated by a period of greater cold. He also showed that this exactly parallels conditions in southern Sweden, as determined by BLYTT.

Somewhat later, VON POST[7] turned his attention to the pollen grains present in the peat, and elaborated methods of pollen analysis. It is argued that the pollen present in the different layers of peat will afford a better picture of the general tree vegetation of the period than is afforded by the remains of wood and fruit, since the pollen is carried by the wind and evenly distributed by chance. By such methods, VON POST was able to give a more detailed picture of Swedish forests during the warm postglacial periods termed "sub-boreal" and "Atlantic" by BLYTT. In the latter period *Quercus*, *Tilia*, and *Ulmus* seem to have been abundant in Sweden. Further confirmatory details were added in a subsequent study,[8] in which regional areas of such postglacial forests were distinguished

[6] SAMUELSSON, G., Scottish peat mosses. Bull. Geol. Inst. Univ. Upsala **10**: 197–260. 1910.

[7] VON POST, L., Skogsträdspollen i sydsvenska torvmosselagerföljder. Geol. Fören. Förhandl. **38**: 384–394. 1916.

[8] VON POST, L., Ur de sydsvenska skogarnas regionala historia under postarktisk tid (Eng. summary). Geol. Fören Förhandl. **46**: 83–128. 1924.

and mapped. It was clearly demonstrated that the mixed oak forest (*Quercus*, *Tilia*, and *Ulmus*) reached its culmination in "Atlantic" time, decreased somewhat in the subsequent "sub-boreal" time, attained another maximum in "early sub-Atlantic" time, and has been steadily decreasing ever since.

The methods used in these studies have been described in detail by ERDTMAN,[9] and more recently by STARK.[10] The peat or silt has often to be boiled in 10 per cent KOH and the residue placed in glycerin for microscopic examination. At times centrifuging may be employed without vitiating the results. The data of the microscopic examination and from the counting of the pollen grains are expressed in pollen diagrams, showing the relative abundance of the pollen of particular species at different horizons of the peat; by pollen spectra showing the relative abundance of pollen of different trees in a particular area; and by pollen maps showing the relative frequencies of different pollens in various areas at any particular time. Directions are given for the construction of such diagrams, spectra, and maps. Among numerous investigations by the same workers, interest seems to center in a preliminary study by ERDTMAN,[11] showing the similarity of the pollen spectra obtained from the peat of southwestern Sweden, northern Scotland, and Ireland,[12] and in a more detailed report on the post glacial forests of northern Scotland. A brief examination of some peat deposits on the Isle of Man[13] reveals a close resemblance in its pollen spectra to those of the early "Atlantic" of Sweden, which are estimated to have been deposited 6500–9500 years ago. Investigations in Denmark by JESSEN[14] also show that its deposits are in accord with the BLYTT-SERNANDER hypothesis of climatic succession. An excellent table here serves to make the various correlations clearly evident. All these results tend to show that in early postglacial times such trees as *Quercus*, *Tilia*, *Ulmus*, and *Fagus* had a much more northern distribution in the British Isles than at present.

ERDTMAN[15] has further shown by the use of similar methods that there was

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[9] ERDTMAN, O. G. E., Pollenanalytische Untersuchungen von Torfmooren und marinen Sedimenten in Südwest-Schweden. Arkiv. Botanik **17**:1–137. 1921.

[10] STARK, P., Der gegenwärtige Stand der pollenanalytischen Forschung. Zeitschr. Bot. **17**:89–125. 1925.

[11] ERDTMAN, O. G. E., Studies in micro-palaeontology I–IV. Geol. Fören. Förhandl. **46**:676–681. 1924.

[12] ———, Studies in the micro-palaeontology of post glacial deposits in northern Scotland and the Scotch Isles, with special reference to the history of the woodlands. Jour. Linn. Soc. Bot. **46**:449–504. 1924.

[13] ———, Pollen statistics from Currogh and Ballaugh, Isle of Man. Proc. Liverpool Geol. Soc. **14**:158–163. 1925.

[14] JESSEN, K., Mose undersoegelser in det nordoestlige Sjaelland (Eng. summary: Bog investigations in northeast Sjaelland). Danmarks Geol. Unders. Series 2. no. 34. 1–241. 1920.

[15] ERDTMAN, O. G. E., On the immigration of some British trees. Jour. Bot. **64**: 71–74. 1926.

an early postglacial increase of *Corylus* pollen in the peat of middle and northern England, showing that it was one of the first forest elements to have reached these regions in its northern migration. It seems to have been followed by *Ulmus, Quercus, Alnus, Tilia,* and *Fagus* in the order named.

Confirmation of these results has come from other portions of Europe. Working in Bohemia, RUDOLPH and FIRBAS[16] found indications of former higher altitudinal limits for forests in the Erzgebirge Mountains. More recently, the same workers[17] investigated the reliability of the pollen analysis hypothesis by examining recent peat deposits in various forest regions. They concluded that, in spite of minor discrepancies, the pollen precipitate at various altitudes reflects with considerable accuracy the composition of adjacent forests. The examination of peat deposits taken at depths up to 240 cm. in the moors of the high Giant Mountains gave pollen diagrams indicating that such trees as *Pinus, Fagus,* and *Quercus* had formerly an altitudinal limit at least 500 m. above that at present existing. This is in agreement with other data indicating that in Bohemia also there was a warmer postglacial period. Similar indications have been obtained as the result of the examination of a fresh water lake silt deposit, termed "Gyttja," and composed almost entirely of organic material. It was taken from marshes representing this former fresh water lake near the southern foot of Erzgebirge Mountains. The author[18] here discusses the possibility of dating these deposits with some accuracy by means of fragments of pottery associated with the pollen in the lacustrine deposits.

That this is possible has been shown by VON POST, using pollen spectra from Scandinavia for dating and synchronizing. A further application comes from the work of WOODHEAD,[19] and of WOODHEAD and ERDTMAN[20] on the peat deposits of the southern Pennines, near Huddersfield, England. Here the pollen diagrams indicate that the lowest of the peat was formed during the warm Atlantic period. Arrowheads and similar remains in this peat are of Neolithic Age. According to DE GEER, the Atlantic period may have lasted for some 3000 years, or about 5200 to 2200 B.C.

These results from the peat deposits seem to warrant interesting data from the application of similar methods in America.—G. D. FULLER.

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[16] RUDOLPH, K., and FIRBAS, F., Palaeofloristische und stratigraphische Untersuchungen böhmischer Moore: Die Hochmoore des Erzgebirges. Beih. Bot. Centralbl. **41**: 1–162. 1924.

[17] ———, Pollenanalytische Untersuchungen subalpiner Moore des Riesengebirges. Ber. Deutsch. Bot. Gesells. **44**: 227–238. 1926.

[18] RUDOLPH, K., Pollenanalytische Untersuchungen in thermopilem Florengebiet Bohmens: Der "Kommerner See" bei Brux. Ber. Deutsch. Bot. Gesells. **44**: 239–248. 1926.

[19] WOODHEAD, T. W., The age and composition of the Pennine Peat. Jour. Bot. **62**: 301–304. 1924.

[20] WOODHEAD, T. W., and ERDTMAN, O. G. E., Remains in the peat of the southern Pennines. Naturalist 245–253. 1926.

Taxonomic notes.—SMALL,[21] in connection with his investigations of the flora of Florida, has discovered a new species of *Lupinus* and of *Polygala*. It is interesting to note that of the 27 species of *Polygala* growing naturally in Florida, about 12 are endemic.

In continuation of his studies of West Indian plants, BRITTON[22] has published descriptions of 21 new species from Cuba, in 18 genera; 10 new species from Trinidad, in 9 genera; and a new tree from Porto Rico.

In presenting what are called the *arvensis*-series of British species of *Viola*, DRABBLE[23] recognizes 10 species, 2 of which are described as new.

In continuation of his description of the GOSSWEILER collection of plants from Angola and Portuguese Congo, GOOD[24] has described a new genus (*Appunettia*) of Rubiaceae, and also 9 new species.

SMALL[25] has described a new species of *Peperomia* (*P. floridana*) from Florida. He states that this "large tropical genus has crossed the Gulf Stream sparingly and settled in Florida in 5 species."

COCKERELL[26] has described a new genus (*Phenacocladus*) of algae from the Green River Eocene Rocks of the Roan Mountains, Colorado. It resembles certain marine forms, but occurred in a lake. COCKERELL concludes that the waters of the lake were saline, and that "the Green River aquatic fauna and flora were remote descendants of a group of marine organisms that had become isolated."

GARDNER[27] has begun a series of publications describing new species of Rhodophyceae from the Pacific coast of North America. The first number contains descriptions and plate illustrations of 10 new species in the following genera: *Helminthora* (2), *Helminthocladia* (1), *Myriogramma* (1), *Erythroglossum* (2), *Membranoptera* (4).

REHDER[28] has published the third part of his presentation of the ligneous plants of northern China, including Leguminosae to Sapindaceae. It presents

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[21] SMALL, J. K., Two new species from Florida. Torreya **26**:91–93. 1926.

[22] BRITTON, N. L., Studies of West Indian plants. XIII. Bull. Torr. Bot. Club **53**:457–471. 1926.

[23] DRABBLE, ERIC, Notes on the British pansies; the *arvensis*-series. Jour. Bot **64**:263–271. 1926.

[24] GOOD, R., Mr. JOHN GOSSWEILER's plants from Angola and Portuguese Congo. Jour. Bot. Suppl. **64**:25–32. 1926.

[25] SMALL, J. K., An additional species of *Peperomia* from Florida. Torreya **26**:109. 1926.

[26] COCKERELL, T. D. A., An alga from the Eocene of Colorado. Torreya **26**:114. 1926.

[27] GARDNER, N. L., New Rhodophyceae from the Pacific coast of North America. I. Univ. Calif. Publ. Bot. **13**:205–226. *pls. 15–21*. 1926.

[28] REHDER, ALFRED, Enumeration of the ligneous plants of northern China. III. Jour. Arnold Arboretum **7**:151–227. 1926.

full details of bibliography and stations, and also the distribution of the species beyond northern China. Much the largest family is the Leguminosae, including 80 species, 4 of which are new, the only new species (*Caragana* and *Wistaria*) described in the paper. The 16 families presented include 52 genera and 185 species, much the largest genera being *Evonymus* (29 species), *Caragana* (22 species), *Acer* (21 species), and *Lespedeza* (13 species). The contribution is a very full and up-to-date record of these families for that region.

JOHNSON[29] has concluded that *Saxifraga Nuttallii* Small should be made the basis of a new genus, to which he gives the name *Cascadia*, for the Cascade Mountains. Its habitat is the Willamette Valley in Oregon and the region bordering on the lower Columbia River.

REHDER[30] has described a new genus (*Monimopetalum*) of Celastraceae from China, which is closely related to *Euonymus*.

In his sixth paper describing new plants from Central America, STANDLEY[31] describes 13 new species in 10 genera. The most interesting one is *Parmentiera Valerii*, which is "a large tree belonging to a group hitherto represented in Central America by only two known species, both of which are trees much inferior in size to this one." Only one tree was seen, and "this was too large to be climbed, and it was only by throwing sticks at the high crown that imperfect specimens of the leaves could be obtained." This is a good illustration of some of the difficulties of collecting.

COOK and HUBBARD[32] have described 5 new species of *Gossypium* from the west coast of South America, in Colombia and Ecuador. They state that the native cottons of that region are not closely related to Mexican species, and also show characters not previously recognized in the genus.—J. M. C.

Trees and shrubs of Mexico.—STANDLEY[33] has published the fifth and final installment of the *Trees and shrubs of Mexico*. This volume brings together all the published species of woody Mexican plants, based on the series of Mexican plants in the National Herbarium. As stated in the preface, "the flora of Mexico, especially the arborescent flora, includes many species of great economic value," and for this reason the presentation includes not merely the taxonomic details, but much information concerning commercial and local uses of the plants. Since it is an assemblage and organization of material rather than a

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[29] JOHNSON, A. M., The status of *Saxifraga Nuttallii*. Amer. Jour. Bot. **14**:38–43. *figs. 2*. 1927.

[30] REHDER, ALFRED, *Monimopetalum*, a new genus of Celastraceae. Jour. Arnold Arboretum **7**:233. 1926.

[31] STANDLEY, P. C., New plants from Central America. VI. Jour. Wash. Acad. Sci. **17**:7–16. 1927.

[32] COOK, O. F., and HUBBARD, J. W., New species of cotton from Colombia and Ecuador. Jour. Wash. Acad. Sci. **16**:545–552. 1926.

[33] STANDLEY, P. C., Trees and shrubs of Mexico (Bignoniaceae—Asteraceae). Contrib. U.S. Nat. Herb. **23**:1313–1721. 1926.

monograph, very few new species are described, only twenty in this last part. Several collaborators have contributed certain groups in which they have specialized.

In the present part 8 families are presented, representing 205 genera and 1327 species of woody Mexican plants. Far the largest family is the Asteraceae (Compositae), with 112 genera and 972 species. The Rubiaceae are second in abundance, being represented by 46 genera and 196 species. There are also 40 pages of "additions and corrections," bringing the information up to date. The extent of these additions and corrections indicates the great activity of our collectors and taxonomists. The volume is a most valuable compendium of information in reference to the Mexican flora.—J. M. C.

Vegetation illustrated.—More than 20 years ago there appeared the first issues of the *Vegetationsbilder*, whose illustrations established a new standard of excellence in depicting the vegetation of various regions. The most recent issues, beginning the seventeenth volume, continue the same high quality of illustrations. The first fascicle[34] gives some of the typical vegetation of the Crimea, and includes woodlands in which are found *Juniperus excelsa*, *Pinus laricio*, and *P. Pithyusa*, together with mountain plateaux, steppes of *Artemisia maritima*, and salt meadows. In another issue[35] the steppes and semidesert of parts of Russia are described and illustrated, while in a third[36] the coast vegetation of southern Dalmatia is depicted. This last shows *Beta maritima* growing wild on the Adriatic coast, and includes among the species illustrated *Capparis rupestris*, *Allium ampeloprasum*, *Inula candida*, *Centaurea Fridrici*, *Alyssum leucadeum*, *Helichrysum italicum*, *Pistacia lentiscus*, and *Euphorbia dendroides*.—G. D. FULLER.

Forest trees of Hokkaido, Japan.—This fine series by MIYABE[37] and his colleagues is presenting the trees of northern Japan in most attractive form. The present fascicles continue the beautiful colored plates already commended,[38] depicting the various stages of the trees from seedling through foliage and flowers to fruit. The four fascicles now issued include *Fagus Sieboldi*, *Castanea crenata*, *Quercus dentata*, *Q. magnolica*, *Q. crispula*, *Q. glandulifera*, *Ulmus japonica*, *U. laciniata*, *Celtis Bungeana* var. *jessoensis*, *Morus bombycis*, and *Cercidiphyllum japonicum*.—G. D. FULLER.

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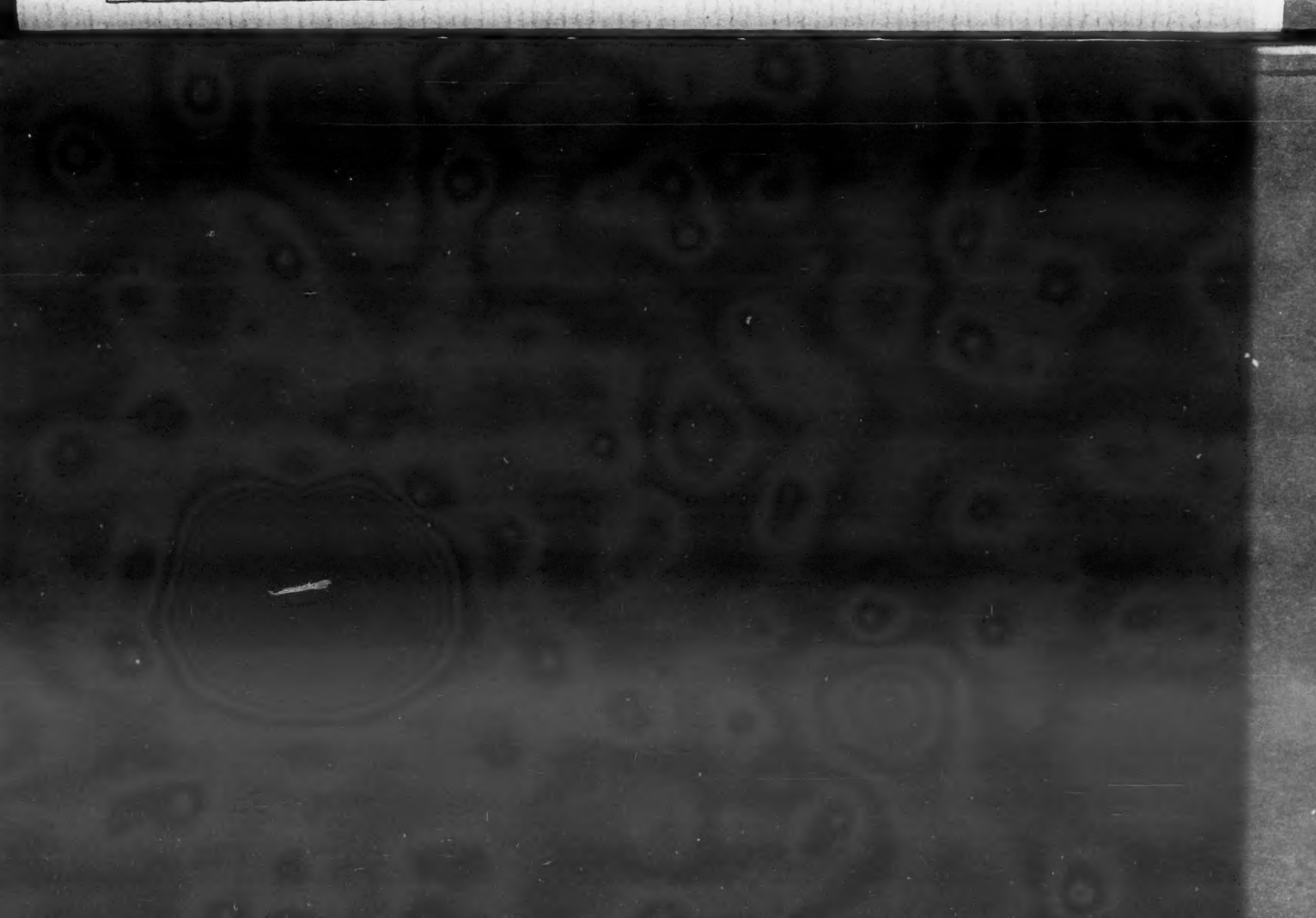
[34] WULFF, EUGEN, Vegetationsbilder aus der Krim. Vegetationsbilder, Karsten & Schenck **17**:Heft 1. *pls. 1–6.* 1926.

[35] KELLER, B., Die Grassteppen im Gouvernement Woronesh (Russland). Vegetationsbilder, Karsten & Schenck **17**:Heft 2. *pls. 7–12.* 1926.

[36] GINZBERGER, A., Küstenvegetation der Süddalmatinischen Eilande. Vegetationsbilder, Karsten & Schenck **17**:Hefte 3, 4. *pls. 13–24.* 1926.

[37] MIYABE, K., and KUDO, Y., Icones of the essential forest trees of Hokkaido. Publ. by Hokkaido Government, Sapporo. **2**:Fasc. 11. 1–6. *pls. 32–34.* 1925; Fasc. 12. 7–14. *pls. 35–37.* 1925; Fasc. 13. 15–22. *pls. 28–40.* 1926; Fasc. 14. 23–26. *pls. 41–43.* 1926.

[38] BOT. GAZ. **72**:55. 1921; **75**:431. 1923.



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